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Links between abnormal lipid metabolism and inflammation in Alzheimer's disease

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Links between abnormal lipid metabolism and inflammation in Alzheimer's disease

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Résumé

La recherche sur la maladie d'Alzheimer (MA) est concentrée, en grande partie, sur l'étude de ses principales caractéristiques histologiques, les plaques β-amyloïdes (Aβ) et les enchevêtrements neurofibrillaires. Cependant, les thérapies ciblant directement ces caractéristiques n'empêchent pas la progression de la MA. En plus de ces caractéristiques, la génétique a mis en évidence l'implication du métabolisme des lipides et de la réponse immunitaire dans la MA. Les perturbations du métabolisme lipidique est le prédicteur génétique le plus puissant du développement de la MA, mais ses mécanismes restent un mystère. Des travaux récents dans notre laboratoire ont montré que les triglycérides s'accumulent dans le cerveau des patients atteints de MA et des souris 3xTg, un modèle murin de la MA. Chez les souris 3xTg, ces triglycérides sont enrichis en acide oléique (AO), un acide gras monoinsaturé, et l'inhibition de l'enzyme de synthèse de l'AO, le stéaryle-CoA désaturase (SCD), réduit leur accumulation et contrecarre la perte précoce de la neurogenèse hippocampique et les troubles de mémoire. Nous avons donc testé si l'inhibition de la SCD peut inverser les changements dans le transcriptome et rétablir la fonction de l'hippocampe chez les souris 3xTg symptomatiques. En comparant aux souris contrôles, l'hippocampe de souris 3xTg possède des altérations transcriptomiques impliquées dans les processus reconnus pour être perturbés dans la MA. Leur hippocampe a également montré une baisse significative des épines dendritiques. De manière remarquable, les données de séquençage de l'ARN montrent que le traitement des souris 3xTg pendant un mois avec un inhibiteur de la SCD a sauvé des gènes liés à l'immunité et aux synapses. Les analyses tissulaires ont révélé que ce traitement a conduit à des améliorations de la densité des épines dendritiques. Nous avons également établi un modèle de microglie en culture et nos données préliminaires suggèrent que les oligomères Aβ pourrait être responsable de perturbations du métabolisme des lipides chez les microglies. En somme, ces études soulignent le potentiel d'un nouveau médicament ciblant SCD pour le traitement de la MA.

Mots-clés

Maladie d'Alzheimer, métabolisme, lipide, microglie, inflammation, synapse, stéaryle-coA désaturase

Summary

Alzheimer's disease (AD) research has mainly focused on studying its main histological hallmarks, β-amyloid (Aβ) plaques, and neurofibrillary tangles. However, therapies directly targeting these hallmarks do not prevent AD progression. In addition to these hallmarks, genetics have highlighted the implication of lipid metabolism and immunity in AD. Disturbances in lipid metabolism are the single strongest genetic predictor of developing AD, but the underlying mechanisms remain poorly understood. Recent work in our laboratory showed that triglycerides accumulate in the brains of both AD patients and 3xTg mice, a mouse model of AD. In 3xTg mice, these triglycerides are enriched with monounsaturated fatty acid oleic acid (OA), and the inhibition of the OA-synthesizing enzyme stearoyl-CoA desaturase (SCD) reduced their accumulation and counteracts the early loss of hippocampal neurogenesis and memory deficits. Here, we tested whether SCD inhibition can reverse changes in the transcriptome and rescue hippocampal function in symptomatic 3xTg mice. Compared to their strain controls, the hippocampus of middle-aged, pre-plaque 3xTg mice showed transcriptomic alterations involved in processes recognized to be disrupted in AD. Their hippocampus also displayed significant reduction in dendritic spines. Remarkably, RNA sequencing data show that treatment of middle-aged 3xTg mice for one month with an SCD inhibitor rescued genes related to immunity and synapses. Tissue analyses revealed that this treatment led to improvements in dendritic spine density. We also established a model of microglia in culture and our preliminary data suggest that Aβ oligomers may be responsible for disruptions in microglial lipid metabolism. Together, these studies shed light on the potential of a novel drug target SCD for the treatment of AD.

Keywords

Alzheimer's disease, metabolism, lipid, microglia, inflammation, synapse, stearoyl-CoA desaturase

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Abbreviations

Aβ: β-amyloid

ABCA7: ATP-binding cassette sub-family A member 7

Acetyl-CoA: Acetyl-coenzyme A

AD: Alzheimer's disease

AICD: Amyloid precursor protein intracellular domain

APOE: Apolipoprotein E

APP: Amyloid precursor protein

APS: Ammonium persulfate

Arg1: Arginase 1

ATP: Adenosine triphosphate

AβO: β-amyloid oligomers

BACE1: β-site amyloid precursor protein cleaving enzyme 1

BBB: Blood-brain barrier

BDNF: Brain-derived neurotrophic factor

BIN1: Bridging Integrator-1

C1q: Complement component 1q

C3: Complement component 3

CAA: Cerebral amyloid angiopathy

CA1: Cornu ammonis

CD: Cluster of differentiation

cDNA: Complementary deoxyribonucleic acid

CLU: Clusterin

CNS: Central nervous system

Cr3: Complement receptor 3

CSF: Cerebrospinal fluid

CSF1: Colony Stimulating Factor 1

CTF: C-terminal fragment

CX3CR1: CX3C chemokine receptor 1

DAM: Disease-associated microglia

DAMP: Damage associated molecular pattern

DAP12: DNAX-activating protein of 12 kDa

DEG: Differently expressed gene

DHA: Docosahexaenoic acid

DIV: Days *in vitro*

DMEM: Dulbecco's Modified Eagle Medium

DMSO: Dimethyl sulfoxide

E: Embryonic day

ECM: Extracellular matrix

EPA: Eicosapentaenoic acid

ER: Endoplasmic reticulum

FA: Fatty acid

FAD-EOAD: Familial Alzheimer's disease- Early-onset Alzheimer's disease

FAO: Fatty acid oxidation

FBS: Fetal bovine serum

FC: Flow cytometry

FDR: False discovery rate

GFAP: Glial fibrillary acidic protein

GO: Gene ontology

GSEA: Gene set enrichment analysis

GWAS: Genome-wide association study

HBSS: Hanks' Balanced Salt Solution

HEXβ: Hexosaminidase subunit β

HFIP: Hexafluoro isopropanol

Hif1α: Hypoxia-inducible factor 1α

HMGCR: 3-hydroxy-3-methylglutaryl-CoA reductase

IBA1: Ionized calcium-binding adaptor molecule 1

IFNγ: Interferon γ

IGF1: Insulin growth factor 1

IL: Interleukin

iNOS: Inducible Nitric oxide synthase

LD: Lipid droplet

LPS: Lipopolysaccharide

LTP: Long-term potential

M0: Homeostatic/resting microglia

M1: M1 pro-inflammatory microglia

M2: M2 anti-inflammatory microglia

mAβ: Monomeric β-amyloid

MHC II: Major histocompatibility complex II

mRNA: Messenger ribonucleic acid

MUFA: Monounsaturated fatty acid

NADP+: Nicotinamide adenine dinucleotide phosphate

NFT: Neurofibrillary tangle

NGF: Nerve growth factor

NLR: NOD-like receptor

NLRP3: NOD-like receptor family pyrin domain containing 3

NO: Nitric oxide

OA: Oleic acid

OXPHOS: Oxidative phosphorylation

P/S: Penicillin Streptomycin

P2RY12: P2Y purinergic receptor 12

PAMP: Pattern associated molecular pattern

PBS: Phosphate buffer saline

PBS-T: PBS-Triton

PFA: Paraformaldehyde

PL: Phospholipid

PS1: Presenilin 1

PS2: Presenilin 2

PUFA: Polyunsaturated fatty acid

RNA: Ribonucleic acid

RNA-seq: Ribonucleic acid sequencing

ROS: Reactive oxygen species

RT- qPCR: Reverse transcriptase quantitative polymerase chain reaction

SAD-LOAD: Sporadic Alzheimer's disease- Late-onset Alzheimer's disease

SCD: Stearoyl-Coenzyme A desaturase

scRNA-seq: Single-cell Ribonucleic acid sequencing

SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SFA: Saturated fatty acid

SLC2A5: Solute carrier family 2-member 5

SNP: Single nucleotide polymorphism

SORL1: Sortilin Related Receptor 1

SPM: Specialized pro-resolving lipids mediator

SREB1c: Sterol Regulatory Element Binding Protein-1C expression

TAG: Triacylglyceride

TEMED: Tetramethyl ethylenediamine

TGFβ: Transforming growth factor β

TGN: Trans-Golgi network

Th: T helper cell

TLR: Toll-like receptor

TMEM199: Transmembrane protein 119

TNFα: Tumor necrosis factor α

TREM2: Triggering receptor expressed on myeloid cells 2

VEGF: Vascular endothelial growth factor

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Introduction

1.0 Alzheimer's disease

Over 50 million people worldwide are living with dementia (10). Alzheimer's disease (AD) is the most common form of dementia. In North America, AD is ranked amongst the top 10 leading causes of death (11). This neurodegenerative disease is mainly characterized by the abnormal protein aggregation and accumulation of β-amyloid (Aβ) and tau. AD is a progressive disease affecting brain function. There are two forms of AD, familial and sporadic AD, also known as early-onset (FAD-EOAD) and late-onset (SAD-LOAD) respectively, the latter being the predominant form. Generally, SAD-LOAD symptoms arise in patients over the age of 60 while symptomatic FAD-EOAD patients are much younger. Though this disease affects both sexes, 2-in-3 people with AD are women (10). Memory deficits are the most known symptom in AD, but it also entails impairments of other cognitive and intellectual functions, such as learning, language, visuospatial skills, behavior, and emotion. As brain function declines, patients will gradually lose their ability to perform day-to-day tasks causing patients to become dependent on their family and caregivers. The medical expenses for individuals with AD was estimated at $10.4 billion in 2016. This is expected to rise to $16.6 billion by 2031 (12).

Diagnosis for AD often occurs late-onset as symptoms are delayed, around 5 to 10 years after irreversible damages to the brain have been initiated (13). A patient presenting with memory deficits will be subjected to a variety of exams complemented with brain imaging to assess structural atrophy, particularly in the hippocampus, the key region affected in AD which is responsible for learning and memory. Clinical assessment of dementia is 85 to 90% accurate due to the complexity and heterogeneity of AD (10). However, AD can only be diagnosed with certainty with an autopsy of the brain. In 2011, the National Institute on Aging-Alzheimer's Association workgroups published recommendations further increasing the accuracy of AD diagnosis in living patients by promoting measurements of biomarkers in the cerebrospinal fluid (CSF) and by positron emission tomography imaging for Aβ and tau proteins (14). Nevertheless, improvements in AD diagnosis are still required as only 10 to 50% of people living with AD are diagnosed (10).

There is currently no cure for AD. Current treatments of AD aim to delay and manage cognitive symptoms. AD patients are provided with cholinesterase inhibitors which increase the levels of

neurotransmitters, such as acetylcholine, to promote brain activity. Clinical trials for AD have been mainly targeting the main events of this neurodegenerative disease including senile plaques and neurofibrillary tangles (NFTs). However, no treatment has been proven to modify the AD course (15, 16). With its increasing prevalence and burden, there is an urgency to identify the mechanisms leading to the initiation and development of AD to promote the establishment of new therapies.

1.1 Risk factors

The risk factors for AD are categorized as non-modifiable and modifiable risk factors. Non-modifiable risk factors include genes, age, and sex while modifiable risk factors are associated with environmental factors and lifestyle choices (figure 1) (17-19). In the past decade, there has been an emerging interest in modifiable risk factors of AD, particularly diet, exercise, and education. Although modifiable factors may not fully prevent the emergence of AD, they can contribute to reducing the risk of AD and delaying its onset.

1.1.1 Genes

There are two forms of AD: SAD-LOAD and FAD-EOAD. They differ in the implication of heritable genetics. FAD-EOAD is a rare form of AD which is dictated by genetics in an autosomal dominant manner. Patients with FAD-EOAD will develop symptoms at early onset, around mid-life in the third or fourth decade of life. The mutations responsible for the increased risk of FAD-EOAD are found in the genes encoding proteins implicated in amyloid precursor protein (APP) processing: *APP*, *presenilin-1* (*PS1*), and *presenilin-2* (*PS2*). APP is cleaved by γ-secretase which contains presenilins, the core catalytic subunits of γ-secretase, to give rise to Aβ peptides. The mutations on these genes modify APP processing resulting in alterations in Aβ production and aggregation. Inheritance of mutations on the *PS1* gene account for a higher proportion of FAD-EOAD (20). It is also important to note that patients with Down syndrome have an increased risk of developing AD as they possess an additional copy of chromosome 21 on which the *APP* gene is located.

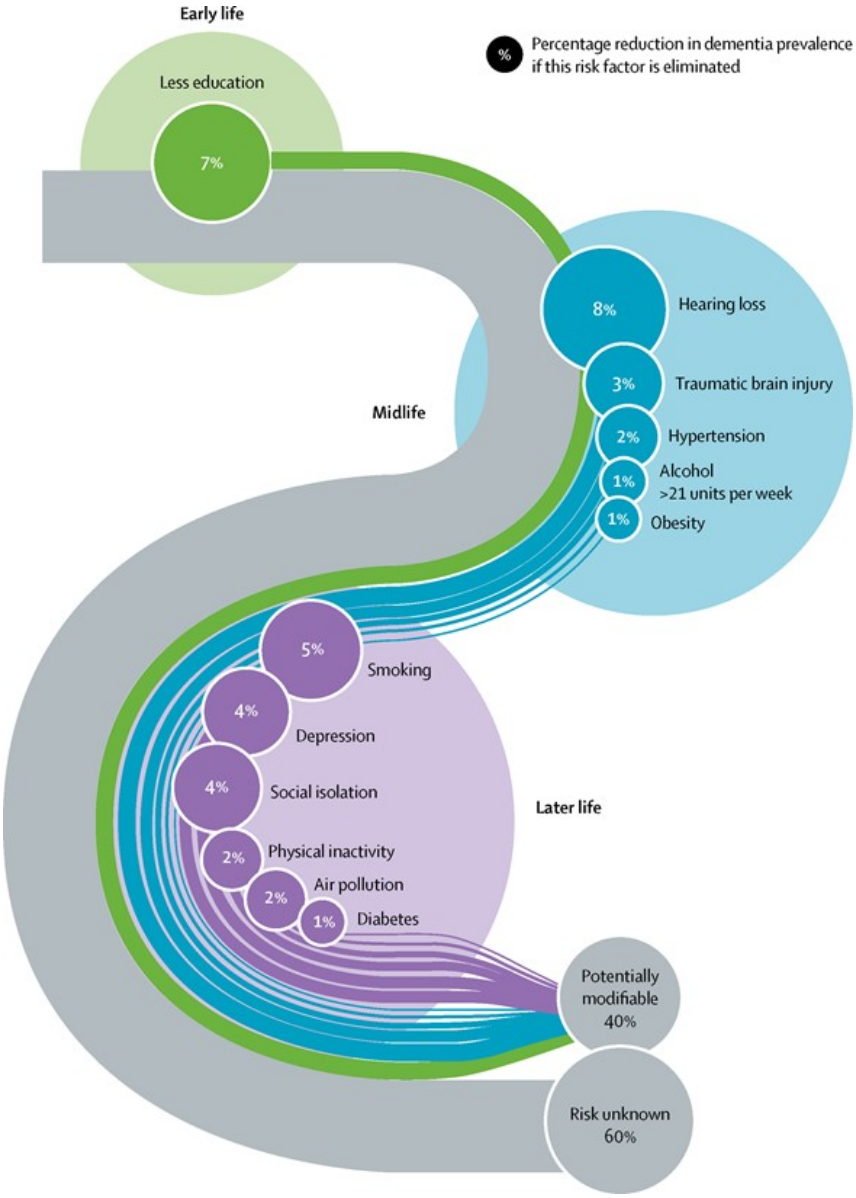


Figure 1. Population attributable fraction of potentially modifiable risk factors for dementia (Reproduced with permission from Elsevier (18))

Regarding SAD-LOAD, it is the predominant form of AD in which symptoms begin to occur at the age of 60 years old or older. Contrary to FAD-EOAD, SAD-LOAD is not hereditary but rather caused by an interplay between environmental factors, lifestyle choices as well as genetic polymorphisms. The *Apolipoprotein E* (*APOE*) gene is the strongest known genetic risk factor for SAD-LOAD. There are three isoforms of APOE: ε2, ε3, and ε4. The carriers of the *APOE ε4* allele are 2- to 15-fold more likely to develop SAD-LOAD while the carriers of *APOE ε2* seem to be protected (5). In addition to *APOE*, genome-wide association studies (GWAS) have identified up

to 40 risk loci in SAD-LOAD patients involved in Aβ and tau pathology, immunity, lipid metabolism, and endocytosis (1-4). The risk genes identified are associated with altered APP processing. These studies highlight the genetic profile of AD, however, identifying the biological processes and function of these loci are important to expand our knowledge of underlying mechanisms leading to AD.

1.1.2 Age

Age is the predominant risk factor for AD. The prevalence of AD between the age of 65 to 69 is under 1% (21). This doubles every 5 years after the age of 65 setting the prevalence at around 25% for AD patients aged 85 years old and older (21). Age at onset is highly associated with AD genetics. FAD-EOAD is attributable to mutations in the APP gene and in the genes of participating in its processing. Patients with FAD-EOAD will develop symptoms around mid-life. The age at onset of SAD-LOAD is 60 years old and older. A study of the effect of GWAS AD risk loci on age identified 10 loci associated with changes in age at onset and revealed that *APOE* was mostly responsible for variations of age at onset (22). Moreover, many hallmarks of aging overlap with AD, such as mitochondrial dysfunction, oxidative damage, aberrant neuronal network activity, stem cell exhaustion, and glial cell activation and inflammation (23). These events are accelerated and amplified by an unknown trigger that has yet to be identified.

1.1.3 Sex

AD affects both men and women. However, the incidence of AD is higher in women (10) and they display a greater cognitive deficit compared to men (24). It was also hypothesized that women are more affected by AD than men due greater life expectancy and to the differences in brain composition between men and women. The male brain has been reported to have a higher brain volume and a higher content of white matter while women have more grey matter (25). This would attribute a certain resilience to brain atrophy in men thus delaying the appearance of cognitive symptoms. As for the severity of AD symptoms, women display greater deficits in cognition compared to men (24). A similar trend is also observed in mouse models of AD (26-29) in which AD progression is also accelerated (30, 31).

Additionally, hormones were also shown to be a contributing factor. Women's susceptibility to developing AD has been associated with the fluctuations of estrogen levels. Estrogen has been reported to enhance cognition and to possess protective effects on the brain (32, 33). The reduced

levels of estrogen in early menopause are associated with the risk of AD (34). Furthermore, *APOE ε4* allele female carriers are more likely to develop AD than their male counterpart (5, 35). Compared to men with a 2-fold risk with one *APOE ε4* allele, women with similar APOE genetics have a 4-fold increased risk (5). Although, it is important to note that homozygous *APOE ε4* allele carriers possess a 15-fold risk of developing AD regardless of sex (5).

1.1.4 Environmental factors and lifestyle choices

a. Diet

Diet is an important pleiotropic factor in health. Nutrition provides important nutrients to the brain, such as lipids, glucose, and amino acid, which are necessary for its normal function and maintenance of neuronal structures. The Western diet which is rich in saturated fats and carbohydrates has been known to cause an imbalance of energy metabolism promoting obesity, cerebrovascular diseases, cardiovascular diseases, and diabetes. Interestingly, these diseases are comorbidities and risk factors for AD. The Western diet owes its detrimental effects to its components, high levels of saturated fatty acids (SFAs) and sugars, which induce inflammation and oxidative stress. In animal models of AD, this diet was even shown to worsen the amyloid burden (36, 37). The Mediterranean diet which is rich in high unsaturated fats and antioxidants seems to possess a protective effect for AD. Mainly composed of polyphenols, monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs), this diet has anti-inflammatory and neuroprotective properties (38-41). The consensus suggests that the Western diet promotes AD while the Mediterranean diet is beneficial for cardiovascular and brain health. The modulation of dietary patterns has been gaining interest in the past decade as a preventive measure and a non-invasive therapy for AD.

b. Exercise

A sedentary life leads to a reduction in lifespan and an increased risk of developing vascular pathologies, cancer, impairment of metabolism, and neurodegenerative diseases. Exercise, particularly aerobic and moderate-to-severe intensity activities, has been reported to reduce the risk of AD (42, 43). An active lifestyle can protect from depression, cardiovascular diseases, and metabolic pathologies, such as hypertension, diabetes, and obesity, which increase the likelihood to develop AD. Physical exercise has been shown to support brain health by increasing the production of growth factors and neurotrophins such as brain-derived neurotrophic factor (BDNF),

insulin-like growth factor 1 (IGF1), and vascular endothelial growth factor (VEGF) which stimulate neurogenesis, cerebrovascular health, neuronal survival, and plasticity as well as to contribute to the brain's energy metabolism (44-47). Interestingly, the levels of these factors were shown to be reduced in AD patients (48). Like diet, the interest in exercise has been on the rise as a potentially safe and non-invasive method of prevention against AD. Furthermore, as diet and exercise closely interact, a combined strategy can possess a synergic effect on brain health.

c. Education

Low educational attainment has been shown to increase the risk of AD. The risk is doubled in people with eight or fewer years of education (49, 50). A meta-analysis showed an inverse dose-response trend between years of education and AD risk leading to a 7% decrease in risk of developing AD per year of education (51). Though the mechanism by which education reduces the likelihood of developing AD is not well understood, education contributes to cognitive stimulation and cognitive reserve (49). The cognitive reserve hypothesis dictates that individual differences in brain resilience and adaptability leads to susceptibility to brain damage and neurodegenerative diseases. A study linked education as a predictor of two AD symptoms: visuospatial ability and working memory (52). Remarkably, intellectual stimulation was demonstrated to reduce the amyloid burden in AD patients (53, 54).

1.2 Hallmarks

1.2.1 Abnormal protein aggregation and accumulation

In 1906, Dr. Alois Alzheimer described cognitive deficits and histological anomalies in the brain of his patient (55). His paper published in 1911 documented the first depictions of Aβ plaques and neurofibrillary tangles (NFT) (56). Nowadays, these observations are known as AD hallmarks which are, still today, the primary neuropathologic criteria for AD diagnosis. A staging system was established to characterize the distribution of NFT (57) in the brain during AD while The Thal phase (58) and CERAD score (59) were established to visualize spatial-temporal distribution of amyloid depositions and to measure dense core plaques, respectively. Nonetheless, the aberrant aggregates of these proteins follow a similar pattern of migration in the brain starting at the entorhinal cortex to the isocortex leading to hippocampal and cortical atrophy as well as enlargement of ventricles (57-59).

a) Aβ depositions

The Aβ protein is derived from APP, a type I transmembrane protein. Although the roles of APP are unclear, it seems to play a role in neuronal development, neurite outgrowth, and axonal transport alongside intracellular Aβ (60, 61). Astrocytes and neurons are responsible for Aβ synthesis. There are two proteolytic pathways for APP processing, the non-amyloidogenic, and the amyloidogenic pathway (62, 63). In the non-amyloidogenic pathway, APP is first cleaved by α-secretase producing soluble APPα and C-terminal fragment (CTF) α which are then cleave by γ-secretase to give rise to P3 and APP intracellular C-terminal domain (AICD). As for the amyloidogenic pathway, APP is cleaved by β-secretase, particularly β-site amyloid precursor protein cleaving enzyme 1 (BACE1), producing soluble APPβ and CTFβ which is then cleaved by γ-secretase generating AICD and 38 to 43 amino acid Aβ. In physiological conditions, APP is predominantly cleaved by α-secretase (64). The most common species of Aβ are $A\beta_{1-40}$ and $A\beta_{1-42}$ (65). In normal conditions, $A\beta_{1-40}$ is abundantly and continuously produced while the latter is produced at a lower level (66). The levels of Aβ in the brain are shaped by Aβ production and clearance. In AD, there is an imbalance between the production of $A\beta_{1-42}$, the principal component of amyloid plaques, via the amyloidogenic pathway and its clearance by phagocytosis or by drainage through the CSF and the brain's vasculature (67). $A\beta_{1-42}$ is commonly described as a "sticky" protein due to its high tendency to self-aggregate. The monomers of this hydrophobic molecule aggregate into soluble oligomers to insoluble fibrils which are resistant to proteolytic cleavage, and ultimately, extracellular amyloid plaques as diffuse or dense core plaques (68).

Aβ oligomers (AβO) are mainly responsible for amyloid neurotoxicity in AD (69). Although plaques occupy a larger surface in the brain, AβO also can impair memory (70, 71) and inhibit LTP (72) in the absence of plaques or neuronal loss. Though fibrillar Aβ also possesses detrimental effects on neurons, the degree of AβO-associated cognitive impairment and inflammation is higher (69, 73, 74). Remarkably, AβO in the brain was reported to induce metabolic deficits in the periphery (75). Additionally, AβO can interact with lipids within lipids rafts of neurons and alter membrane integrity to form pores (76). Pore formation causes an imbalance in calcium homeostasis leading to cell death by promoting mitochondrial dysfunction and apoptosis. Aβ can also disrupt mitochondrial bioenergetics by eliciting a chronic inflammatory response or by directly interfering with the electron transport chain and oxidative phosphorylation thus reducing adenosine triphosphate (ATP) production and promoting the production of reactive oxygen species

(ROS) and nitric oxide (NO) (77). Moreover, AβO can bind to multiple receptors downregulating cell survival, such as the nerve growth factor (NGF) receptor, the N-methyl-D-aspartate receptor, and the insulin receptor (78, 79). More importantly, as impairment of cognition correlates with loss of synapses, amyloid's role in AD-associated synaptotoxicity has been studied. AβO prompts changes in dendritic structure and morphology (80-82) as well as the loss of synapses (81, 83-86). This phenomenon is due to the excitotoxic effects of oligomers causing alterations in synaptic plasticity and synapse inactivation by targeting glutamatergic receptors (87). Altogether, Aβ is heavily implicated in various AD pathology.

b) Tau species

The tau protein is a microtubule-associated protein encoded by the MAPT gene. Tau is a dynamic molecule vital for the assembly and stability of tubulin regulating cytoskeletal dynamics. This protein is mainly found in the axons of neurons and is important for synaptic trafficking. In humans, there are six isoforms of tau produced by alternative splicing in exons 2,3, and 10. Abnormal inclusions of tau are not limited to AD. They have also been reported in frontotemporal dementia with parkinsonism-17, Pick disease, progressive supranuclear palsy, cortico-basal degeneration, argyrophilic grain disease, and chronic traumatic encephalopathies. The intracellular tauopathies in AD are found as NFT in neurons and neuropil threads in dendrites and axons which are composed of all six isoforms of tau. There are also extracellular accumulations of tau called ghost tangles. In AD, tau phosphorylation is 4-fold higher than in healthy brains (88). The hyperphosphorylation of tau leads to reduced affinity for microtubules and prone to aggregate into straight or paired helical filaments, the latter being the primary component of NFT in AD (89).

Tau's neurotoxic effects are exerted by its intracellular and extracellular accumulation. The intracellular accumulation of tau occurs in the somatodendritic compartment and axons of neurons. Cell death is induced as axonal transport and microtubule network are disrupted (89). Tau can also destabilize mitochondrial dynamics by increasing fission and decreasing fusion causing increased production of ROS and impaired production of ATP (90). Extracellular tau possesses a neurotoxic effect on calcium homeostasis, neurite pathology, and vesicle trafficking. Tau can bind muscarinic receptors on neurons and induce mobilization of calcium ions leading to neuronal cell death (91). Although the effect of tau on dendritic and synaptic morphology and density is unclear, it is required for AβO synaptotoxicity (92, 93).

1.2.2 Cerebral amyloid angiopathy

Aβ depositions can also be found in blood vessels disrupting the brain's vasculature (94). This is known as amyloid cerebral angiopathy (CAA) which can manifest as strokes and progressive dementia. There are two forms of CAA, hereditary and sporadic with the latter being the most common. Like AD, patients with CAA can possess neuritic plaques (95). Although the mechanisms inducing CAA have not been established, mutations in *APP* and *PS1* can give rise to CAA (96). The *APOE ε4* allele is also a risk factor for sporadic CAA (97, 98). Contrary to AD, CAA associated with increased levels of $A\beta_{1\text{-}40}$ aggregates and are mostly found in arteries, arterioles, and capillaries of cerebral and cerebellar cortices and leptomeninges (99). The clinical presentations of CAA are also distinct from AD manifesting as spontaneous intracerebral hemorrhage and transient neurological focal episodes. Nonetheless, the similarities between AD and CAA suggest a mechanistic link.

1.2.3 Gliosis

Gliosis refers to the activation and proliferation of glia in response to brain injury, to an insult by pathogens, or neurodegenerative-associated proteins. This phenomenon occurs in aging and CNS injury as a defense and regeneration mechanism. The cells involved are astrocytes and microglia (100) which closely interact together to maintain a healthy and functional brain network. Astrocytes play a vital role in tissue scarring and blood-brain-barrier maintenance (BBB) reconstruction after an injury as well as in the immunomodulation of microglia. In AD, Aβ aggregates and hyperphosphorylated tau species can trigger sustained reactive astrogliosis and microgliosis resulting in neuronal death, chronic inflammation, altered dendritic morphology, demyelination, and the inhibition of axon regeneration. Microglial activation stimulates pro-inflammatory cytokines production and secretion to activate a cascade of pathways designed to rid the brain of pathogens and debris. In chronic reactive microgliosis, these cytokines are constantly produced and induce neuronal atrophy (101). As for astrogliosis, its sustained activation leads to excitotoxic glutamate effects on neurons and the breakdown of the BBB (102). Stimulated astrocytes can also trigger microglial activation further contributing to neuroinflammation and neuronal stress. Moreover, chronic activation of astrocytes and microglia enhances production of ROS and NO largely contributing to oxidative stress in AD.

1.2.4 Lipid accumulation

Alongside his observations of amyloid plaques and NFT, Dr. Alzheimer also reported abundant "lipoid granules" in the brain of his AD patient (55, 56). As studies have greatly focused on amyloid and tau pathologies, lipid metabolism has been overlooked. Brain lipid imbalances play a major role in neurodegenerative diseases, neurological disorders, and stroke (103). It manifests as neuronal energy collapse and accumulations of lipid droplets (LDs). These lipid accumulations were observed in humans (104) and mouse models of AD (104, 105). Also, the predominant AD risk gene, *APOE*, plays a major role in lipid transport in the brain. Altogether, these studies emphasize the role of aberrant lipid metabolism in AD. Yet, the mechanisms linking lipid metabolism to AD pathologies remain poorly understood.

1.3 Physiopathology

1.3.1 Amyloid cascade hypothesis

The amyloid hypothesis was introduced in the early 1990s (106, 107). It postulates that the accumulation of Aβ aggregates is the cause of the AD physiopathology. The evidence supporting this hypothesis resides in the genetic aspects of AD along with Aβ's detrimental effects on the brain. AD have been associated with mutations in genes implicated in aberrant amyloidogenesis. Also, Aβ species trigger a cascade of changes in the brain by promoting sustained inflammation, neuronal atrophy and cell death. To further verify this hypothesis, studies have sought to determine if the addition of Aβ can induce AD-like pathology and whether removing Aβ from AD brains is a potential treatment. In mouse models of AD, the introduction of mutated human APP with and without PS1 results in Aβ pathology and induces cognitive deficits as early as 4 months of age (108) suggesting that Aβ induces AD. However, in humans, Aβ pathology and AD do not posses a direct causal relationship. Studies have reported the presence of amyloid depositions in the absence of cognitive affections (109-111). Therefore, the degree of amyloid burden is not proportional to cognitive impairment. Yet, in normal elders and AD patients, the presence of amyloid depositions accelerates brain atrophy (112-114). As for the benefits of Aβ removal on cognition, clinical trials fail to cure AD via the reduction of $A\beta_{1-42}$ levels as they show no improvement in cognition (15, 16). However, these clinical trials recruit patients that already display irreversible damages which allows for one only refutable conclusion: alleviating the amyloid burden in late-stage AD does not rescue the underlying symptoms or change AD course. More work is required to study the role of Aβ in the early stages of AD.

1.3.2 Tau propagation hypothesis

In AD, the tau propagation hypothesis was introduced in 2009 (115). It posits that abnormal hyperphosphorylated tau aggregates induce protein misfolding of normal tau causing proteins to aggregate in a prion-like manner and to propagate throughout the brain. Tau propagation in the brain is well documented and correlates with AD progression and cognitive decline (57). However, it is reported that similar to Aβ, some individuals with NFT do not have cognitive affections (116). The accumulation of abnormal tau aggregates impairs synaptic transmission, mitochondrial function, and axonal transport leading to synaptic loss and neuronal cell death. Clinical trials have targeted tau aggregation by modulating kinases and phosphatase as well as by increasing microtubule stability, but they have all failed to stop AD progression (15, 16).

In addition to Aβ and tau pathology, genetic have highlighted the implications of lipid metabolism and immunity in AD (1-4). These processes represent interesting targets for AD. *APOE ε4* allele (5) and microglial *triggering receptor expressed on myeloid cells 2* (*TREM2*) gene (6, 7) represent the top two genetic risk factors of AD. As recent studies demonstrate the importance of alterations in lipid metabolism and immunity in AD, we investigate the potential role of lipid metabolism and inflammation in AD development and progression.

2.0 Lipids in the brain

Lipids account for close to 50% of the brain's dry weight (117). Brain lipids are comprised of four main species: triacylglycerols (TAGs), fatty acids (FAs), phospholipids (PLs), and sterol lipids. Lipids are essential for brain function serving as energy storage compartments, signaling molecules, and structural components of cell membranes. These important macromolecules are found abundantly in the lipid rafts of cell membranes and the myelin sheath of axons. Most lipids come from diet and are transported into the brain from the systemic circulation through the BBB by passive diffusion. The primary sites of lipid synthesis are the adipose tissue and the liver. Oxidative tissues, such as skeletal muscle and the heart, also produce lipids to a lesser extent. Lipids are stored in the cell as LDs which are mostly composed of esterified cholesterol and TAGs. LDs act as an energy storage unit, signaling molecules, and a defense mechanism from mitochondrial lipid toxicity when lipids are in excess in the cytoplasm.

2.0.1 Fatty acids

FAs are classified by the length of their carbon chain: short (<6 carbons), medium (6–12 carbons), and long (>14 carbons). They are also categorized as SFAs, MUFAs, and PUFAs depending on the number of carbon-carbon double bonds. The brain can perform *de novo* synthesis of SFAs and MUFAs via TAG lipolysis or acetyl-CoA through glucose metabolism. However, the brain depends on diet as a source of essential omega-6 PUFA linoleic acid and omega-3 PUFA α-linolenic acid. FAs can directly enter β-oxidation once transformed to FA acyl-CoA to fuel energy requirements or they can be stored by being added to the glycerol backbone of glycerophospholipids.

a) Saturated fatty acids

SFAs possess a linear carbon chain without carbon-carbon double bonds. They originate from diet and *de novo* synthesis. SFAs are converted to MUFAs of the same length by stearoyl-CoA desaturase (SCD) which introduces a double bond at the 9^{th} carbon of the fatty acid chain. The most abundant SFAs are palmitic and stearic acid. SFAs have been reported to play a role in the regulation of food intake and energy consumption (118). However, the consumption of an SFA-rich diet is associated with an increased risk of cardiovascular diseases (119) and Alzheimer's disease (120). They have mostly been associated with deleterious effects, such as accumulation of body fat, hyperphagia, insulin resistance, glucose intolerance, and inflammation. Palmitic acid, in particular, is a potent activator of microglia (121, 122) and was also shown to induce metabolic reprogramming (123).

b) Monounsaturated fatty acids

Brain MUFAs stem from brain *de novo* synthesis and the diet. They possess one carbon-carbon double bond on their carbon chain. SFAs and MUFAs have often been shown to have opposing effects. Though diets enriched with MUFAs lead to fat mass accumulation similar to SFAs (124), they have been shown to reverse the effect of an SFA-rich diet. Studies have shown that MUFAs can prevent palmitic acid-induced apoptosis and glucose intolerance in the pancreas (125). Oleic acid (OA), the most abundant MUFA, was shown to improve insulin resistance induced by SFAs (124, 126-128). Additionally, OA has been associated with anti-inflammatory properties (127, 129, 130).

c) Polyunsaturated fatty acids

Other well-established fatty acids with anti-inflammatory properties are omega-3 PUFAs, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). These fatty acids possess multiple carbon-carbon double bonds on their fatty acid chain, and they come from dietary fats. They possess important functions in the brain, including neurogenesis, neuronal differentiation, neuroprotection, synaptogenesis, and neurotransmission (131). PUFAs also possess highly potent anti-apoptotic, anti-oxidative, and anti-inflammatory abilities as well as potential benefits on age-associated pathologies (132). DHA and EPA as well as their specialized pro-resolving lipids mediators (SPMs) are essential for the attenuation of inflammation in microglia and astrocytes following brain injury and insult from pathogens (133-135). However, not all PUFAs are beneficial. Omega-6 arachidonic acid exert opposite effect to DHA like increase inflammation and reduced synaptic signal transduction (136). Studies have shown that a decrease in DHA and a rise in arachidonic acid in circulation were observed in subjects with depression and anxiety (137, 138).

2.0.2 Phospholipids

PLs can be segregated into two major classes: sphingolipids and glycerophospholipids. These lipids are predominantly found in cell membranes closely interacting with cholesterol modulating cell membrane integrity, permeability, and flexibility (139). Hence, their important role in vesicular budding and membrane fusion. They allow for protein anchoring into the membrane and dynamic membrane remodeling. Sphingolipids are classified into two main groups glycosphingolipids which are enriched in the grey matter as well as in neurons and sphingomyelin which is found in oligodendrocytes and myelin (140). Ceramides are also a major sphingolipid. They participate in cellular processes including autophagy, apoptosis as well as cell death, and proliferation. As for glycerophospholipids, there are major forms including phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine. These molecules compose the inner leaf of plasma membranes modulating their curvature and fluidity (139). Phosphatidylcholine and phosphatidylserine act as reservoirs for choline, an essential component of neurotransmitters, and DHA, respectively.

2.0.3 Sterol lipids

The major form of sterol lipids is cholesterol. In the brain, it is mostly found as an unesterified form. Cholesterol synthesis occurs by the mevalonate pathway starting with acetyl-CoA. Glia, such as astrocytes, produce cholesterol via the Bloch pathway while neurons will favor the Kandustch-Russel pathway (141). Although all cells in the brain can produce cholesterol, astrocytes are the principal endogenous source of cholesterol in the brain. In the periphery, the liver produces most of the sterol lipids found in the body which are loaded into lipoproteins to traverse the BBB. In the brain, APOE is the predominant lipoprotein. It plays an important role in the transport and distribution of PLs and cholesterol. Excess cholesterol is stored in LDs. In addition to its role in the cell membrane and myelin, cholesterol is crucial for the synthesis of neurotransmitters and hormones as well as neurite growth and axonal guidance.

2.1 Lipid metabolism in the brain

Although the main source of energy in the brain is glucose, lipids contribute to approximately 20% of its energy requirement through FA oxidation (FAO) (142). This phenomenon occurs predominantly in astrocytes which provide metabolic support to neurons. Metabolites such as ketones, acetyl-coenzyme A (acetyl-CoA), flavin adenine dinucleotide, cholesterol, and lactate are produced in astrocytes which are then taken up by neurons. As neurons are unable to store important pools of energy, they are highly dependent on astrocytes. Astrocytes also protect hyperactive neurons from lipid toxicity shuttling LD to their mitochondria (143). Microglia also act as a protector of neurons from lipid toxicity through the phagocytosis of the surplus of LDs. Moreover, oligodendrocytes are also important cells in brain lipid metabolism. They are responsible for the production of myelin sheaths for axons. Altogether, lipids are important for the structural and functional maintenance of the brain.

2.2 Role of lipid metabolism in Alzheimer's disease

2.2.1 Abnormal lipid metabolism in Alzheimer's disease

Lipid dyshomeostasis has been associated with numerous neurodegenerative diseases, including AD, Huntington's disease, Niemann–Pick Type C disease, and Parkinson's disease. The major lipid classes known to be disrupted in AD include cholesterol, sphingolipids, and FAs (144-148). Disturbances in lipid metabolism are primarily observed as abnormal accumulations of LDs (104, 105).

a) Lipid raft: Cholesterol and sphingolipids

The major component of lipid rafts, cholesterol, and sphingolipids were found to be altered in AD. Elevated cholesterol levels in midlife were associated with an increased risk of AD (149). An AD-like phenotype can be induced in mice with a diet enriched with cholesterol (150). In the brain of AD subjects, high levels of cholesterol were measured (151, 152) and this was also replicated in animal models of AD (153, 154). Studies have reported that cholesterol promotes Aβ production through the amyloidogenic pathway (see "*Role of lipids in pathological processing of APP*") and tau hyperphosphorylation (155, 156). Remarkably, a study also reported a correlation between high cholesterol levels and NFT burden in the hippocampus and cortex (157). Clinical trials on statins, inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) the limiting enzyme to cholesterol *de novo* synthesis, for AD treatment yield conflicting results. Some indicate no significant effect on cognition (158, 159) while others report improvement of cognition (160, 161).

As for sphingolipids, the implications of sphingolipids in AD have not been investigated in-depth compared to cholesterol. Sphingomyelin is metabolized by sphingomyelinase to produce ceramides. Sphingomyelinase activity was discovered to be enhanced in AD which concurs with findings that found ceramide to be increased while sphingomyelin was depleted (162-164). Elevated levels of ceramide have been associated with increased risk of AD, and lowering its levels is associated with cognitive improvements (165). Also, they participate in pathological Aβ production (see "*Role of lipids in pathological processing of APP*").

b) Fatty acids

Alterations in FAs are strongly associated with AD. These FAs include SFAs palmitic acid as well as unsaturated FAs linoleic acid, linolenic acid, DHA, EPA, OA, and arachidonic acid (146, 148). Here we will discuss the implications of major lipid species palmitic acid, OA, and DHA on AD physiopathology.

Palmitic acid

Palmitic acid was found to be increased in AD brains (146). It was reported to induce cellular stress resulting in increased BACE1 activity (166-168) and hyperphosphorylation of tau (168). Additionally, palmitic acid is a strong inducer of microglial activation via Toll-like receptors (TLRs) (121, 122)and was shown to modify the phagocytic and migratory activity of microglia in response to stimuli (169, 170)). In obese mice, microglial activation by palmitic acid was shown

to impair synaptic plasticity and correlate with memory deficits (171). Hence, palmitic acid assists in cellular stress and inflammation in AD.

Oleic acid

A rise in OA levels was observed in AD brains (144-146). Though OA is praised for its benefits related to the Mediterranean diet, excess of OA can also be harmful. The inhibition of OA production protected mice from obesity (172, 173) and insulin resistance (174-176). A *in vitro* study demonstrated that cells treated with OA increased expression of PS1 and secretase activity resulting in a rise in Aβ levels (177). Interestingly, work in our lab demonstrated that OA was enriched in the brain of a mouse model of AD negatively impacting neuronal stem cell activity (104). By inhibiting the OA-synthesizing enzyme SCD neuronal stem cell impairment was rescued. Thus, the overproduction of OA via SCD seems to contribute to lipid dyshomeostasis in AD.

Docosahexaenoic acid

Many studies have highlighted the decreased levels of DHA in AD (144, 145, 147, 178, 179). Although the cause for reduced DHA in the brain has not been identified, a study explored the effect of APOE on DHA transport to the brain. They showed that *APOE ε4* mice appears to possess a reduced brain uptake of DHA compared to *APOE ε2* mice (180). A DHA-rich diet has protective effects against mild cognitive impairment and AD (120, 181). In mouse models of AD, DHA plays an important role in dendritic pathology and cognitive decline. It was shown to rescue spine loss (133, 182) and ameliorate memory (182, 183). Clinical trials on DHA supplementation as a treatment for AD-associated cognitive alterations resulted in conflicting results with a study showing improvements (184) and another observing no effects (185). As previously mentioned, DHA possess inflammatory pro-resolving effects on microglia and astrocytes (133-135). They are precursors of SPMs, crucial lipids for resolution of inflammation, and wound healing. Remarkably, these lipids were also found to be reduced in the entorhinal cortex and hippocampus of AD patients (186, 187). The reduction of essential DHA and its derivatives impairs brain function potentially contributing to AD progression.

2.2.2. Lipid-related risk genes of Alzheimer's disease

GWAS studies have identified several lipid genes associated with increase susceptibility to SAD-LOAD (1-4, 188). Single nucleotide polymorphisms (SNPs) in the major candidate genes were

found in the loci for lipid-related proteins including APOE, ATP-binding cassette sub-family A member 7 (ABCA7), Clusterin (CLU), Bridging integrator 1 (BIN1), and Sortilin Related Receptor 1 (SORL1).

a. APOE

In the brain, APOE is the predominant lipoprotein. It is responsible for lipid transport, particularly cholesterol, between cells in the brain. APOE is mainly expressed by astrocytes but under certain conditions, neurons, ependymal cells, and microglia can also produce APOE. As previously mentioned, *APOE* is the strongest known genetic risk factor for SAD-LOAD. While the carriers of the *APOE ε4* allele are up to15-fold more likely to develop SAD-LOAD, carriers of *APOE ε2* seem to be protected (5). APOE isoforms were also observed to have a similar effect on the risk of developing CAA (97, 98).

APOE is implicated in various aspects of AD, including Aβ pathology, tauopathy, inflammation as well as cell metabolism. *APOE ε4* promotes Aβ seeding in early AD (189) and Aβ oligomerization (190). It also increases the amyloid burden in AD brains (191). The depletion of APOE resulted in reduced Aβ deposits (192, 193). *APOE ε4* was also shown to facilitates tau hyperphosphorylation (194). As for neuroinflammation, *APOE ε4* patients displayed a higher proportion of reactive microglia than other APOE isoforms (195). Enriched in amyloid plaque, APOE facilitates the uptake of Aβ by microglia via TREM2 (196, 197). The TREM2-APOE pathway mediates microglial activation (30) and its changes associated with neurodegenerative diseases (8, 9). It was also shown to contribute to dendritic spine loss in AD (198).

Furthermore, lipid and glucose metabolism were found to be disrupted in an APOE isoform dependent manner. Farmer et al demonstrated that *APOE ε4* astrocytes display smaller but significantly more LDs than their *APOE ε3* counterparts (199). Smaller APOE particles were also observed in the CSF of *APOE ε4* carriers (200). Lipidation was also less efficient in *APOE ε4* subjects (201). Finally, the brain of AD patients exhibited reduce glucose metabolism (202).

b. ABCA7

In the brain, *ABCA7* is highly expressed particularly in the cortex and the CA1 of the hippocampus. It is found to be abundant in microglia and neurons. ATP-binding cassette proteins are important lipid transporters for PL and cholesterol and are responsible for the translocation of these lipids to cell membranes as well their efflux from cells to APOE and Apolipoprotein A. ABCA7 is also a

key regulator of lipid homeostasis via modulation of cholesterol levels. Two main variants of *ABCA7* were identified for SAD-LOAD, rs3752246, and rs3764650, associated with its loss of function. ABCA7 can modulate phagocytosis in macrophages and microglia. The phagocytic activity of these immune cells in response to Aβ was dampened by ABCA7 knockout (203). Sakae et al also found that *ABCA7* deletion alters Aβ biosynthesis by promoting the increased expression of BACE1 thus resulting in increased Aβ production (204). Consistent with the reduction in clearance of Aβ and enhanced processing, the absence of ABCA7 leads to an increase in amyloid burden in mouse models of AD (204) and humans (205, 206). This protein's role in tauopathy has been studied to a lesser extent. A study reported that *ABCA7* variants were associated with decreased Aβ levels in the CSF but did not influence tau or hyperphosphorylated tau levels (207).

c. CLU

Also known as Apolipoprotein J, *CLU* is constitutively expressed in all tissues. It plays an important role in apoptosis, complement activation, cellular stress immune modulation, and lipid transport. CLU key functions to act as an extracellular chaperone. In the brain, astrocytes are the principal source of CLU production although neurons can also produce CLU in cases of brain injury, neuroinflammation, and neurodegenerative diseases. CLU seems to play a key role in lipid accumulation and metabolic disorders as it can regulate Sterol Regulatory Element Binding Protein-1C expression (SREB1c), a crucial regulator of several lipid metabolic pathways (208). In AD, there are three main SNPs in *CLU* that were identified: rs11136000, rs2279590, and rs9331888 (209). CLU and APOE are the main transporters of cholesterol and Aβ in the brain. Some have reported that CLU is beneficial for AD by inhibiting aggregation of Aβ and enhances clearance (210) while another report that CLU contributes to Aβ toxicity (211). Though CLU was found to be upregulated in the frontal cortex and hippocampus, studies suggest that that the ratio CLU: Aβ dictates it is neuroprotective or neurotoxic (212, 213). Also, a proportional relation between CLU and tau levels was reported in the CSF (214) and the brain of AD patients (215).

d. BIN1

Mostly associated with vesicular endocytosis, *BIN1*, also known as Amphiphysin 2, is a member of the Bin/Amphiphysin/Rvs family of adaptor proteins. It is capable of binding to the lipids within the cell membrane to modulate membrane curvature thus regulating lipid membrane dynamics. In human AD brains, *BIN1* SNP rs59335482 (216) and rs744373 (217) are associated with an

increased tau burden. A recent study demonstrates a mechanism by which BIN1 modulates the mislocalization of tau into synapses induce synaptic dysfunction and spine loss (218). Furthermore, BIN1 was shown to promote extracellular tau and its phosphorylated species seeding via extracellular vesicles and it hinders microglial clearance of tau (219). Knockdown of BIN1 in Drosophila attenuated tau but not Aβ mediated neurotoxicity (204). The role of BIN1 on Aβ pathology is conflicting. A study reported that decreased BIN1 leads to a rise in BACE1 levels increasing Aβ production (220) while others report no effect on APP processing nor on the amyloid burden. (216). Essential for tubulation and cytoskeleton remodeling, BIN1 mediate AD tauopathy more consistently than amyloid pathology.

e. SORL1

Also knows as SorLA and lipoprotein receptor 11, SORL1 regulates endosomal and trans-Golgi network (TGN) transport. It is a member of the low-density lipoprotein receptor family of APOE receptors and the vacuolar protein sorting 10 domain receptor family. Mostly enriched in neurons, SORL1 plays an important role in APP processing and Aβ production. It was found to colocalize with APP in the TGN and early endosomes. In AD brains, SORL1 was shown to be reduced in SAD-LOAD and not in FAD-EOAD (221, 222). *SORL1* has neuroprotective abilities by reducing APP cleave and consequently, Aβ production. Many mechanisms were proposed to explain SORL1's role in APP processing. One mechanism suggests that SORL1 expression induces redistribution and mobilization of intracellular APP to the TGN (223) while the second mechanism implies that SORL1 utilizes retromer complexes to redirect APP to the TGN inhibiting entry into endosomes where the first steps of APP cleavage occur (224).

2.2.3 Role of lipids in pathological processing of Amyloid precursor protein

APP is synthesized in the endoplasmic reticulum (ER) and is translocated through the Golgi apparatus to the TGN for sorting. APP is then loaded into endosomes or vesicles and trafficked to the cell membrane to be cleaved by secretases to produce extracellular Aβ species. If the APP is not processed, it is returned to the TGN or sent to the ER via retromers. Lipids actively participate in APP processing, particularly cholesterol, sphingolipids, and DHA.

During the amyloidogenic pathway, the cleavage of APP by β-secretase takes place within cholesterol- and sphingomyelin-enriched lipids raft in the cell membrane. Cholesterol is crucial for the surge in Aβ production via the amyloidogenic pathway by boosting BACE1 and γ-secretase

proteolytic activity (155, 225, 226) along with promoting BACE1 sequestering to lipid rafts (227). Cholesterol was also shown to play a crucial role in Aβ aggregation (228). Studies have shown that preventing cholesterol production via inhibition of HMGCR (229) and depletion of cholesterol (230) reduces Aβ production. Thus, statins have been employed as a potential treatment for AD. However, clinical trials on statins for AD yield mixed outcomes. Some indicate no significant effect on the amyloid burden (231, 232) while others report alleviation of the amyloid burden (233).

Regarding sphingolipids, the key regulators of membrane fluidity, a study demonstrated that heightened fluidity promotes the non-amyloidogenic processing of APP via enhanced α-secretase activity and impaired APP internalization (234). In AD, sphingolipids, such as ceramide, were found to be increased while sphingomyelin is decreased (162-164). These two lipids possess opposing roles in APP processing. While ceramides stimulate Aβ production, sphingomyelin constrains its production (235). Interestingly, Aβ can modulate lipid homeostasis via regulation of lipid enzymes. Studies by Grimm et al demonstrate that $A\beta_{1-40}$ is capable of suppressing HMGCR activity thus lowering cholesterol levels while $A\beta_{1-42}$ activates sphingomyelinase to reduce sphingomyelin synthesis to heighten its production via ceramides (225, 235).

Finally, DHA promotes the non-amyloidogenic pathway. This occurs by inhibiting BACE1 activity, enhancing α-secretase stability, and lowering cholesterol via the inhibition of HMGCR (236, 237). Also, DHA lowers levels of PS1 (238). Altogether, lipids and Aβ species closely interact modulating key components of their synthesis.

Lipids are vital for brain function, but its processes must be tightly regulated to prevent imbalances and deleterious effects on the brain. Alterations in lipid metabolism promote AD but the underlying mechanisms remain poorly understood. Recent studies have highlighted the crosstalk between lipid metabolism and immunity and their role in modulating inflammation and dictating microglial phenotypical conversion to disease-associated microglia (DAM) or microglia neurodegenerative phenotype (8, 9). In the following section, we explore the emerging role of lipid metabolism in microglia dysfunction in AD.

3.0 Microglia

Microglia are the primary immune cell in the CNS. They represent 12% and 16% of cells in the CNS in mice and humans, respectively (239, 240). Derived from erythromyeloid precursors cells, they originate from the yolk sac appearing in the neuronal tube at early stages of embryogenesis around embryonic day 9 (E9) in mice (241, 242) and around the 5th week of gestation in humans (243). Although they share many functions, microglia are quite distinct from macrophages with diverging transcriptomic profiles and origins (244, 245). Transcriptomic signatures uniquely expressed in microglia include transmembrane protein (TMEM)119, solute carrier family 2-member 5 (SLC2A5), P2Y purinergic receptor (P2RY)12, β-hexosaminidase subunit β (HEXβ). Although microglia are not derived from fetal hematopoietic stem cells like macrophages (246), these stems cells can contribute to the microglial pool during pathological events (247, 248). Microglia sustain self-renewal via apoptosis and local proliferation. Microglial proliferation and survival require colony-stimulating factor 1 (CSF1) (249) and transforming growth factor-beta (TGFβ) (250). *In vitro* astrocytes nurture microglia by providing these factors as well as cholesterol (251). CSF1 was shown to be particularly important as its inhibition leads to impaired microglial proliferation and cell death (249, 252, 253).

Microglia are highly versatile and adapt to the needs of the CNS. They make up a heterogeneous population in the brain. They are subject to spatial and temporal heterogeneity. Spatial heterogeneity refers to the assortment of microglial populations between different brain regions varying from their panel of immune markers, their transcriptomes, and their functions as well as their density (254-256). As for temporal heterogeneity, microglia assume different transcriptomic and immune profiles according to requirements at different stages of life from the embryo to adulthood (257-260). For example, immature microglia express P2RY12 while TMEM119 is only expressed once microglia are mature (260). During pre-and postnatal development, microglia are active displaying an amoeboid form to facilitate migration and modulate cell death and apoptosis, axonal outgrowth and fasciculation, cortical interneuron migration, neuronal survival, and oligodendrogenesis and synaptic development (261). Microglia maintain the pool of neuronal progenitor cells by contributing to their proliferation, survival, maturation as well as controlling their abundance (262-264). They also closely interact with oligodendrocyte precursor cells promoting myelinogenesis (265). In the developing brain of adolescents, microglia are important for the modeling of neuronal (266, 267) and synaptic circuits (268, 269).

As for adulthood, the ultimate goal of microglia is to maintain the brain's proper function. Microglia are highly perceptive constantly scan the brains for changes in the microenvironment. To maintain a healthy brain microenvironment free of pathogens, misfolded proteins, debris, and apoptotic cells, microglia possess a diversified sensome of receptors. Astrocytes and T cells can also activate microglia via cytokines and chemokines during insults and brain injury. Microglia also monitor neuronal activity, refine synaptic neurotransmissions, and protect the brain from infiltrating invaders (261). Additionally, microglia support neurogenesis in neurogenic niches, hippocampus (266), and subventricular zone (270) throughout life.

Microglial aging occurs in a region-dependent manner (271). During aging, microglia will enter a primed state in which they express higher levels of pro-inflammatory receptors and cytokines in a chronic low-grade manner. This results in an exaggerated inflammatory response when challenged. This phenomenon is also known as "inflammaging" (23, 272). Aged microglia display a reduction in number and length of ramifications, a limited migration ability, and lowered phagocytic activity (273, 274). As microglia play a central role in shaping and maintaining the neural network, their dysfunction has been associated with inducing neurodegenerative diseases.

3.1 Microglial polarization

Microglia possess a broad spectrum of activation. However, they have been commonly classified by their state of activation for simplicity and conceptualization of microglial activities. Hence, M0 for homeostatic microglia, M1 for pro-inflammatory microglia, and M2 for anti-inflammatory microglia. These classes are equally important and equilibrium between M1 and M2 is crucial for proper brain function. Each state of polarization has specific signatures and immune markers (275-278). They can also be distinguished amongst them by their morphology. Homeostatic microglia are highly ramified with thin branches while activated microglia display a reduced number of thick branches or no branching adopting an ameboid form to facilitate migration and phagocytosis (274, 277). As previously mentioned, microglia and macrophages are separate entities though microglial polarization nomenclature stems from macrophage classification. Therefore, there are gaps in knowledge of microglia polarization, and more research is required to fully document microglial responses.

3.1.1 Homeostatic microglia (M0)

Homeostatic microglia are also referred to as resting microglia. However, this term is contradicting as M0 microglia are continuously in movement. As the sentinel of the brain, they are constantly probing and surveilling the brain's microenvironment. The homeostatic phenotype is enriched with TMEM119, P2RY12, SLC2A5, and CX3C Motif Chemokine Receptor 1 (CX3CR1) (259). TGFβ is a potent cytokine modulating microglial activation (250, 279). Neurons also contribute to ensuring that microglia remain homeostatic in absence of an immune challenge. Cluster of differentiation (CD) 200 and CX3C Motif Chemokine Ligand 1 bind to CD200 receptor and CX3CR1 (280), respectively. These mechanisms are required to prevent microglia from going rogue by inhibiting non-elicited activation of microglia.

3.1.2 Pro-inflammatory microglia (M1)

Pro-inflammatory responses are activated via the classical pathway of microglial activation. An immune challenge is triggered when microglia recognize damage-associated molecular patterns (DAMPs), such as ATP, Aβ, α-synuclein, and lipids as well as pathogen-associated molecular patterns (PAMPs), bacterial motifs (i.e., lipopolysaccharide (LPS), peptidoglycan, flagellin) and viral motifs (i.e., double-stranded ribonucleic acid (RNA) and CpG oligodeoxynucleotides). Astrocytes (281) and T helper 1 (Th1) cells (282) can also secrete interferon γ (IFNγ), tumor necrosis factor α (TNFα), interleukin 1β (IL1β), and IL6 to activate microglia. These inflammatory molecules are recognized by the microglia's arsenal of receptors including TLRs, NOD-like receptors (NLRs), C-type lectin receptors, Fc receptors, P2R/X receptors, and scavenger receptors (283). A cascade of transcription factors, such as Nuclear factor-κB, and inflammatory complexes, including the NLR family pyrin domain containing 3 (NLRP3) inflammasome complex, are activated to the secretion of cytokines leading to the initiation of proliferation, migration, apoptosis, and phagocytosis to rid the brain of invading pathogens and to clear cellular debris (284). These cytokines are TNFα, IL1β, IL6, IL12, IL23, and NO (275-277). Microglia also secrete proteases via complement activation and undergo oxidative burst producing ROS and superoxide anion to further promote the destruction of pathogens (275, 276, 285). Additionally, microglia participate in establishing immune memory. They secrete chemokines to recruit T helper cells and present antigens at their surface via major histocompatibility complex II (MHC II). M1 cells will upregulate the expression of MHC II, integrins, co-stimulatory molecules, and CD68, a transmembrane glycoprotein protein linked with lysosomal activity (275, 276, 285). Microglia are

the warriors of the CNS. However, the proteins secreted by microglia to eliminate intruders also have harmful effects on neurons. Hence, the importance of M2 anti-inflammatory microglia which counteract inflammation and promote tissue repair.

3.1.3 Anti-inflammatory microglia (M2)

Anti-inflammatory microglia are known for their role in neuroprotection. Known to be activated by the alternative pathway of inflammation, its functions are to resolve microglial pro-inflammatory responses as well as to restore homeostasis following clearance of debris and elimination of pathogens. This phenotype can be induced by three mechanisms: (a) through cytokines secretion of IL4 and IL13 by Th2 cells, (b) through the activation of specific Fcγ receptors, TLRs, and immune complexes, and (c) through IL10, glucocorticoids, and TGFβ. M2 microglia modulate inflammation through the recruitment of regulatory T cells which suppresses inflammation (278). Anti-inflammatory microglia also produce and secrete cytokines, growth factors, and extracellular matrix (ECM) molecules to promote wound healing and remodeling. Known immune markers for these microglia are IL3, IL10, IL21, IL33, CD206, chitinase-3-Like-3, and arginase 1 (Arg1) (275-278). Arg1 is important for the conversion of arginine into ECM molecules, such as polyamines and ornithine, and it can also downregulate the production of NO (276). M2 microglia will also secrete molecules to promote tissue repair as well as neuronal survival and proliferation, such as IGF1, BDNF, fibroblast growth factor, nerve-derived growth factor, and glial cell-derived neurotrophic factor (275, 276). Microglia also participate in maintaining vascular health through VEGF (275, 276). A lack of M2 and consequently, failure to dampen the actions of M1 microglia have been associated with processes underlying neurodegenerative diseases.

3.2 Microglia metabolism

Glycolysis is primarily responsible for most of the microglia's energy requirements. Glucose enters the cell via glucose transporters and is converted to pyruvate. It is then sent to the mitochondria into the Krebs cycle to produce ATP via oxidative phosphorylation (OXPHOS), also known as mitochondrial respiration. In hypoxic conditions, microglia perform anaerobic glycolysis where pyruvate is transformed into lactate and shuttled via monocarboxylate transporters. Glycolysis generates nicotinamide adenine dinucleotide phosphate ($NAPD^{+}$) necessary for mitochondrial electron transport and mitochondrial respiration. Microglia are

flexible and capable of switching from a source of energy to another to exert their metabolic functions in response to demands and changes in the brain's microenvironment. They can also rely on ketone bodies, FAs, and amino acids for fuel. Homeostatic microglia mostly rely on OXPHOS for their energy metabolism. As they require constant energy to roam the brain, mitochondrial respiration is an efficient and reliable source of ATP. M0 microglia can use glutamine as fuel when glucose is depleted (286). This is accomplished as glutamine is converted to α-ketoglutarate which can enter the Krebs cycle.

As for M1 microglia, they display increase glucose uptake (287-291) and lactate production (287, 289). Pro-inflammatory microglia shift to anaerobic glycolysis by inhibiting OXPHOS and FAO (289). This transition from OXPHOS to glycolysis is known as the "Warburg effect" and it results in the production of hypoxia-inducible factor 1α (HIF-1α) and succinate which drives the production of IL1β (292-294). Though OXPHOS yields an important amount of ATP compared to anaerobic glycolysis, it leads to rapid production of glucose providing a burst of energy for microglia to quickly migrate and exert their biocidal abilities. Glycolysis is essential for the activation of the M1 phenotype. The inhibition of glucose consumption via glucose transporter inhibition (290) as well as limiting glycolysis itself by utilizing a glucose analog completely blocks the activation of a pro-inflammatory response (291, 295). On the other hand, M2 microglia have decreased glucose consumption and lactate production (287) but increased ATP production via OXPHOS (296). FAO is also important for M2 cells to carry out its anti-inflammatory activity (297, 298). They utilize OXPHOS and FAO to promote the transcription of genes associated with tissue repair and the production of growth factors. If OXPHOS is defective, M2 polarization cannot occur and M1 inflammation remain unresolved (299).

3.3 Microglia in Alzheimer's disease

Neuroinflammation is a key factor in many neurodegenerative diseases. In AD, maladaptive microglia response results in exaggerated synaptic pruning, neuronal loss, inhibition of neurogenesis, oxidative stress, demyelination, and BBB permeability leading to cognitive impairment. RNA sequencing (RNA-seq) studies have identified a subpopulation of microglia associated with AD (8, 9, 30). These microglia are referred to as DAM phenotype, microglia neurodegenerative phenotype, and activated response microglia. These studies found that genes associated with homeostasis were found to be downregulated whereas the expression of

inflammatory genes was upregulated in AD (8, 9, 30). Recent findings suggest that the changes observed in microglia occur in response to Aβ (300, 301). Once seen as the hero, microglia become harmful to the brain. In the mouse model of AD, the removal of microglia was shown to produce benefits in AD (252, 253, 302, 303). However, as microglia are also crucial for maintenance and protection, therapies modulating microglia should be privileged.

3.3.1 Microglia in Aβ pathology

Neuroinflammation and Aβ were found to be correlated in the human brains of AD (304). Microglia can be found around amyloid plaques in AD brains (305-307). They possess a variety of receptors to recognize Aβ species and its plaques, such as TLR4, CD33, CD36, TREM2, Fc receptors, and scavenger receptors (283). Aβ pathology in AD is driven by its heightened production and reduced clearance in the brain. Microglia participate in these key processes. In the early stages of AD, acute microglial activation by Aβ leads to its uptake and clearance (101, 308). However, as inflammation promotes amyloidogenesis, pro-inflammatory factors produced by microglia increase APP processing and Aβ production (308-311). Microglial activation by Aβ also gives rise to NLRP3 activation which promotes Aβ seeding (312). Furthermore, exposure to Aβ and systemic inflammation was also shown to prime microglia where subsequent activation leads to disproportionate immune response (313, 314). As the balance between production and clearance is disrupted, microglia are chronically exposed to increasing levels of Aβ leading to increased proliferation and recruitment of microglia as well as increased levels of cytotoxic molecules in the brain. The accumulated Aβ species will aggregate forming oligomers, fibrils, and finally plaques. Alongside amyloid plaques, sustained activation of microglia induces oxidative stress in neurons and exaggerated synaptic pruning. As AD progresses, microglia functions are compromised, and they enter a tolerant state with reduced ability to phagocyte and to engage an immune response to Aβ but maintain a sustained release of pro-inflammatory molecules (315). GWAS studies that identified AD risk genes whose variants are associated with impaired recognition and clearance of Aβ by microglia in AD (3, 188, 316). An emerging concept is maladaptive microglia as the driver for abnormal Aβ aggregation. Many studies have shown that microglia accumulate Aβ and release it back into the brain's microenvironment (317, 318). To further elucidate microglia's role in Aβ pathology, studies have depleted microglia in mouse models of AD. Short-term depletion of microglia via CSF1 receptor inhibition in a mouse model of AD did not alter amyloid burden but it rescued synaptic and neuronal loss as well as memory deficits (253, 302). On the contrary, long-

term depletion of microglia in young mice alleviated amyloid burden and improved cognitive deficits (252, 303). Altogether, microglia play a key role in the development and progression of Aβ-associated AD pathology.

3.3.2 Microglia in tau pathology

In AD brains, activated microglia are found close to extracellular tau species and neurons carrying NFTs (304, 319, 320). Microglia enter the M1 pro-inflammatory state when in contact with tau (321, 322). Recognition of tau species by microglia is mediated by TREM2 and CX3CR1 receptors. Once tau binds to these receptors, clearance of tau by phagocytosis is initiated although microglia possess a moderate ability to internalize tau compared to infiltrating macrophages (323). A study demonstrated that microglia participate in tau seeding in mice (324). This was confirmed when the removal of microglia impaired tau propagation in the early stages of AD (325, 326). However, in later stages of AD, reduced uptake of phosphorylated tau by microglia aid in increasing the NFT burden. TREM2 (327) and CX3CR1 (328, 329) depletion or its disrupted signaling was associated with accelerated and exacerbated hyperphosphorylation and aggregation of tau. Also, chronic microglial activation leads to the sustained production of pro-inflammatory cytokine IL1β which further triggers tau phosphorylation (330). Pathological microglial activation participates in AD tauopathies by promoting phosphorylation and seeding of tau. Altogether, these studies, in addition to the study conducted by Sierksma et al., suggest that neuroinflammation occurs upstream from AD tauopathies.

3.3.3 Excessive synaptic pruning

As previously mentioned, microglia shape synapses throughout life. Synaptic pruning is essential for the specificity of synaptic connections by eliminating excess and weak spines. This phenomenon is modulated by signaling via CX3CR1 and complement receptor 3 (CR3). Complement components 1q (C1q) and 3 (C3) tag synapse destined for microglial engulfment. The refinement of synaptic connectivity is crucial to ensure efficient neuronal communications. Synaptic loss entails shortening of dendrites and a reduction in dendritic spine density. In AD, C1q and C3 levels were upregulated in mice (331, 332). Interestingly, studies have demonstrated that AβO injections in the brain induced upregulation of C3 and C1q levels (333, 334). These studies suggest that AβO induces exaggerated spine engulfment by microglia leading to loss of synapses. In mouse models of AD, inhibition of C1q and C3 rescued cognitive impairment, synaptic loss,

and neuronal loss (270, 334). Moreover, microglial activation was shown to have synaptotoxic effects through the secretion of pro-inflammatory cytokines, such as TNFα, IL6, and NO (335). Hence, through the upregulation of complement factors and activation of microglia, AβO induces loss of synapses. Altogether, this suggests that microglia and AβO closely interact causing an exaggerated synaptic loss in AD.

3.3.4 Immune-related risk genes of Alzheimer's disease

Immune-related risk genes with increased susceptibility to develop SAD-LOAD are mostly associated with microglia immune response. Top candidate risk genes were found in the loci of Triggering Receptor Expressed on Myeloid Cells-2 (TREM2) and CD33 (3, 188, 316). Remarkably, a study by Sierksma et al investigated the contribution of AD risk gene variants to its pathology and they found that these genes were altered in response to Aβ and not to tau. This hints at the significant involvement of Aβ in AD-associated changes in microglia.

a. TREM2

TREM2 is a receptor found on cells of myeloid lineage. In the brain, this transmembrane glycoprotein is exclusively expressed in microglia. TREM2 is important for the clearance of lipids (phospholipids, glycolipids, and lipoproteins), apoptotic neurons, cellular debris, bacterial products, and Aβ. It also mediates microglial proliferation and bioenergetics. TREM2 signaling occurs via a DNAX-activating protein of 12 kDa (DAP12). Mutations in *TREM2* have been associated with an increased risk for neurodegenerative disease (336). In AD, *TREM2* is the second strongest genetic risk factor after *APOE* with a two- to three-fold increased risk (6, 7). Interestingly, TREM2 and the APOE-TREM2 pathway are regulators of microglia polarization in AD (6, 7). Its main variant is the *TREM2* R47H allele (9). It causes loss of function impacting microglia's ability to be activated (9, 337-340) and to bind to its ligands (197, 341-344). Moreover, its levels are elevated in the cortex and hippocampus of AD brains as a form of compensation for its altered function (345).

TREM2's role in Aβ pathology has been largely studied. TREM2 is upregulated in microglia surrounding amyloid plaques (346). However, TREM2 depletion or signaling failure through DAP12 impairs microglial migration to the plaques and accelerates Aβ pathology (344, 347, 348). This was also shown in human AD (9, 347). TREM2 was shown to have different effects on the Aβ burden depending on AD progression. In mouse models of AD, TREM2 deficiency reduced

amyloid plaque in the cortex and the hippocampus (349, 350) but accelerated amyloidogenesis and increased Aβ (347) in the early stages of AD. Regarding late stages of AD, the absence of plaque-associated microglia reduced amyloid plaque compaction leading to increased neuronal dystrophy and plaque size (347, 348). Furthermore, as Aβ uptake is increased when complexed with APOE (197, 351), TREM2 mediates APOE production by microglia (347). Likewise, *TREM2* mutations impair its ability to sense lipids (350). In sum, TREM2 aid microglia in the clearance of Aβ and the formation of a protective barrier for neurons against amyloid plaques. As for tau, similar to Aβ, *TREM2* depletion and its R47H variant play different roles at different stages of AD. In early tauopathy, it protects against microgliosis (339) but promotes tau seeding (324) while in the late stages of AD, TREM2 depletion increases its hyperphosphorylation and aggregation (327). Altogether, TREM2 is protective against abnormal accumulation and aggregation of Aβ and tau and it represents a bridge between lipid metabolism and neuroinflammation.

a. CD33

Also knows as sialic acid-binding immunoglobulin-like lectin 3, CD33 is a transmembrane receptor expressed on peripheric myeloid cells and microglia. This receptor can bind to sialic acid found on proteins as well as pathogens and lipids (apolipoproteins and gangliosides). The activation of this receptor leads to inhibition of microglial activation. It protects microglia from eliciting an immune response from "self" molecules. Remarkably, it was shown that TREM2 and CD33 closely interact and have opposite effects on microglia and amyloid pathology (338). Knockout of TREM2 inhibits microglial pro-inflammatory response whereas knockout of CD33 upregulates pro-inflammatory genes. Griciuc et al also show that TREM2 acts downstream of CD33.

In AD, the major *CD33* variant is rs3865444. It has a protective (minor (A/T) allele) and risk allele (major (C/G) allele). While the protective allele was found to reduce CD33 levels and protect against AD (352), the risk allele displayed opposite effects with increased levels of CD33 and increased amyloid burden (353). In AD, *CD33* risk allele levels were upregulated while the minor allele was decreased (352-354). Additionally, the *CD33* risk allele was also associated with cognitive decline (353, 355). Hence, CD33 expression reduces microglial activation leading to impaired clearance of Aβ and tau as they both present sialic acid residues. The depletion of CD33 increased Aβ uptake by microglia and decreases the amyloid burden but does not affect Aβ

degradation nor its biosynthesis (352). Furthermore, amyloid plaques and NFT are rich in gangliosides and sialic acid that allows them to mask them from microglia by activating the CD33 receptor. Together, studies demonstrate that the CD33 AD risk gene impairs microglial clearance.

3.3.5 Metabolic reprogramming

An emerging field of study in AD is microglia bioenergetics. In AD, chronic activation of microglia in the presence of Aβ leads to impaired clearance of Aβ, increased microglial proliferation, and sustained production of pro-inflammatory cytokines resulting in neuronal death. Although the concept of a link between disrupted energy metabolism and impaired microglia function is relatively new, recent findings suggest that altered metabolism in the AD brain is a key player in microglial dysfunction. In physiological conditions, glycolysis is crucial for the activation of a microglial pro-inflammatory response. However, chronic activation of microglia to Aβ leads to metabolic defects by downregulating OXPHOS and glycolysis and results in decreased phagocytic activity and dampened immune responses (73). This phenomenon was reversed when treating microglia with IFNγ, a mediator of glycolysis, and the mTOR pathway. Interestingly, a study demonstrated that TREM2 variants were associated with altered glycolysis, reduced ATP production, and mTOR signaling as well as defective microglia functions (356). TREM2 was shown to be an important regulator of lipid metabolism in microglia (357). TREM2 deficiency and its loss of function inhibit the activation of microglia and lead to lipid accumulations and neuronal damage (357). Noteworthy, recent work in our lab shows that triglycerides accumulate in the forebrain of both AD patients and AD mice (104). We demonstrate that these triglycerides were enriched with MUFAs, particularly OA which was reported to modify microglial metabolic patterns (297) and weakened microglial response to LPS (127). These studies suggest that microglia undergo metabolic alterations preventing them from engaging an immune response.

The majority of AD risk loci identified by GWAS studies are solely expressed in microglia highlighting their importance in AD pathology. The identification of the TREM2-APOE axis as a regulator for the DAM phenotype suggests a mechanistic link between inflammation and lipid metabolism. As immunometabolism is a promising target for AD, the role of lipids in AD-associated neuroinflammation requires further attention.

4.0 Rational & Hypothesis

In this memoir, I focus on disturbances in brain lipid metabolism, the single strongest genetic predictor of developing AD which remains poorly understood. Previous work in our laboratory has identified the presence of abnormal lipid metabolism manifesting as accumulations of triglycerides in post-mortem brain samples of human AD patients. Interestingly, we also demonstrated that these early-onset accumulations precede cognitive impairment in a transgenic murine model of AD, 3xTg (104). In 3xTg mice, these triglycerides are enriched with monounsaturated OA. The inhibition of the OA-synthesizing enzyme stearoyl-CoA desaturase (SCD) rescued forebrain neural stem cell activity (104) and memory (unpublished). This suggests that excess MUFA contributes to AD pathology and that SCD inhibition is a potential therapeutic target.

4.1 Stearoyl-CoA desaturase

SCD is a crucial enzyme for MUFAs synthesis. It incorporates a cis double bond between carbons 9 and 10 to the acyl-CoA groups of SFAs earning its name Δ-9 desaturase. This enzyme is found bound to the ER membrane. In mice, there are four isoforms of SCD (SCD1, SCD2, SCD3, and SCD4) while humans only express two isoforms (SCD1 and SCD5). In humans and mice, SCD1 is ubiquitously expressed but highly expressed in lipogenic tissues including adipose tissue and the liver. SCD2 is also ubiquitously expressed except in the liver. In the mouse brain, SCD2 expression is particularly high during the neonatal stage of life. As for SCD3, it is mostly present in the skin as well as the Harderian and preputial glands while SCD4 is mainly expressed in the heart. Finally, human SCD5 is found in the pancreas and the brain. SCD1 is the predominant and most studied form of SCD in humans and mice. Its main SFAs substrates are palmitate and stearate which are converted to palmitoleic acid and OA, respectively. SCD1 activity is activated by high carbohydrate and saturated fat feeding. Its expression can also be induced by glucose, fructose, saturated fatty acids, vitamins, and insulin which is achieved through liver X receptors, SREBP-1c, peroxisome proliferator-activated receptors, and CCAAT/enhancer-binding proteins. SCD1 repressors include leptin and PUFAS.

SCD1 plays a crucial role in lipid homeostasis by mediating SFA: MUFA ratio. Alterations in its activity results in disturbances in energy metabolism promoting the development of diseases, such as cancer, diabetes, metabolic syndrome, and obesity (358). SCD1 deficient mice had delayed

tumor growth and cancer cell death (359, 360) and were protected from developing obesity (173, 361), fatty liver (362), glucose tolerance (174, 363), and insulin resistance (174-176). Moreover, as a surge in SCD1 activity induces inflammation (364), its depletion in Agouti mice and mice on a high-fat diet displayed reduce adipocyte and macrophage-mediated inflammation (365). Interestingly, SCD2 was found to be enriched in the mouse hypothalamus and its knockdown induce a reduction in weight as the body's energy expenditure was increased (366).

4.2 Stearoyl-CoA desaturase in Alzheimer's disease

SCD1 expression (144) and OA (144-146) were found to be elevated in the brain of AD patients. Upregulation of SCD1 correlated with poor cognitive performance (144). As previously mentioned, high levels of OA increased amyloidogenic APP processing (367) and impaired neuronal stem cell proliferation which was rescued by SCD1 inhibition (104). Interestingly, SCD1 inhibition was found to be beneficial in another neurodegenerative disease as well. It inhibited α-synuclein-induced lipid dyshomeostasis by impairing α-synuclein toxicity and neuronal degeneration in Parkinson's disease (368). Hence, SCD1 and OA seem to play a key role in neurodegeneration. SCD also plays a role in lipid-related inflammation in the brain, a study also showed that SCD1 depletion reduces the pro-inflammatory phenotype induced by prolonged exposure to the myelin of microglia thus promoting remyelination (369). More importantly, macrophages upregulate SCD1 when treated with Aβ (370). Altogether, SCD1 represents an interesting target for modulation of aberrant lipid metabolism and inflammation in AD.

4.3 Mouse model of Alzheimer's disease

In the following memoir, we conducted experiments with 3xTg mice, a mouse model of AD, and age matched WT strain B6129SF2/J 101045 mice. 3xTg mice were originally derived by co-microinjecting two independent transgenes encoding human Swedish mutant APP and the human tau P301L mutation, both under control of the neuron-specific mouse Thy1.2 regulatory element, into single-cell embryos harvested from homozygous M146V mutant PS1 knock-in mice (371). This animal model represents a model of familial AD as it entails the incorporation of mutated transgenes PSEN1, human APP and the human tau resulting in the overproduction of Aβ and tau and leading to amyloid plaques and NFTs. 3xTg mice is one of the only mouse models of AD that recapitulate both amyloid and tau pathology as well as AD hallmarks such as CAA, gliosis, and lipid accumulation (26, 27). They also display cognitive (26, 27) and metabolic deficits (104, 372).

3xTg display similar AD pathology observed in AD patients compared to other mouse models expressing mutations leading to either Aβ or tau pathology exclusively, such as APP/PS1, 5XFAD and TauΔK280 mice. Additionally, it is important to note that experiments were performed in female mice except for *in vitro* studies, the latter being due to limitations in sexing P1 pups. Compared to males, female 3xTg mice display behavioral abnormalities earlier (31, 373) and with higher severity (31, 374-376). They also present higher severity of Aβ pathology (376-378) tau pathology (376) and gliosis (376). More importantly, Jackson Laboratory reported that 3xTg male mice may not exhibit phenotypic traits of AD.

The goal of this project is to investigate SCD inhibition as a therapeutic approach for intervention in AD. We hypothesized that alterations in lipid metabolism are responsible for changes in microglia immune response and other downstream effects in AD and that SCD inhibition can intervene in these processes. We mainly focus on early stages of AD when 3xTg mice display AD-related changes, such as deficits in neurogenesis (379) and cognition (unpublished) as well as peripheral metabolic alterations (unpublished) prior to the formation of amyloid plaques and NFTs. To test this hypothesis, we established three objectives:

1) To determine the effect of SCD1 inhibition on the transcriptome of the 3xTg hippocampus.

   We performed RNA-seq to profile the genome-wide transcriptional changes in the 3xTg hippocampus and the impact of SCD1 inhibition on these changes.

2) To determine the effect of SCD inhibition on hippocampal dendrites.

   Dendrites and their spines are neuronal processes important for neurotransmission between neurons. In AD, synaptic loss disrupts neuronal connectivity and plasticity leading to cognitive deficits (380, 381). We performed Golgi staining on hippocampal slices of female 3xTg and WT mice that received a 28-day intracerebroventricular infusion of an SCD inhibitor via an osmotic pump. We assessed the effect of SCD inhibition on dendritic arborization as well as dendritic spine density in the hippocampus of middle-aged 3xTg mice and WT control mice.

3) To determine the impact of SCD1 inhibition on microglia-mediated inflammation.

   We first sought out to study microglial polarization in 3xTg mice and WT control mice. We performed flow cytometry on microglia from the whole brain of 2- and 8-month-old female 3xTg and WT control mice to characterize microglial polarization at an asymptomatic and symptomatic age. We also established a primary microglia culture from WT and 3xTg

neonatal pups to study gene expression of microglia in response to immune challenges and the impact of SCD1 inhibition on these responses.

Material and methods

Animal Experimental Models

Experiments were conducted following the guidelines of the Canadian Council of Animal Care and were approved by the institutional animal care committee of the Research Center of the University of Montreal Hospital (CRCHUM). 3xTg-AD mice (stock # 034830) and their WT strain B6129SF2/J (stock #101045) were obtained from Jackson Laboratory mice. Experiments were performed on female 3xTg mice (PS1M146V, APP_{Swe}, and Tau_{P301L}) (371) and their aged matched WT control except for primary microglia cultures in which both sexes were used. Ages are specified in the figure legends. All mice were bred at the CRCHUM and kept in identical housing conditions and given free access to a standard rodent diet (#2018, Harlan Teklad) and water.

Intracerebroventricular injection

Alzet 28-day osmotic pumps (0.11 µl/h infusion rate, model 1004; Durect, cat #0009922) attached to brain infusion cannula (Durect, cat# 0008851) were stereotaxically implanted into the left lateral ventricle (coordinates: 0 mm AP and −0.9 mm ML to the bregma) according to manufacturer's instructions. Pumps contained the vehicle, DMSO (Sigma Aldrich, cat# D2650), or 80µM SCD1 inhibitor (Abcam, cat# ab142089) diluted in DMSO both products were diluted in artificial cerebrospinal fluid according to manufacturer's instructions.

RNA-sequencing

RNA extraction from tissue

Ipsilateral hippocampi were dissected from intracerebroventricular injected mice and immediately flash frozen on dry ice. Total RNA was extracted with TRIzol (Invitrogen cat# 15596018) and chloroform (Invitrogen cat# 15593031) according to the manufacturer's instructions. RNA was then purified with the RNeasy mini kit (QIAGEN cat# 74104) according to the manufacturer's instructions. RNA was quantified using a NanoDrop™ 2000/2000c Spectrophotometer (Thermofisher Scientific). 1µg of total RNA was sent to the Institute for Research in Immunology and Cancer's Genomics Platform (IRIC).

Library preparation and RNA-seq

400 ng of total RNA was used for library preparation. RNA quality control was assessed with the Bioanalyzer RNA 6000 Nano assay on the 2100 Bioanalyzer system (Agilent technologies) and all samples had a RIN above 8. Library preparation was done with the KAPA mRNAseq Hyperprep kit (KAPA, cat#. KK8581). Ligation was made with 38 nM final concentration of Illumina dual-index UMI (IDT) and 11 PCR cycles were required to amplify cDNA libraries. Libraries were quantified by Qubit 2.0 Fluorometer (Thermofisher Scientific) and BioAnalyzer DNA1000. All libraries were diluted to 10 nM and normalized by quantitative PCR using the KAPA library quantification kit (KAPA, cat# KK4973). Libraries were pooled to equimolar concentration. Sequencing was performed with the Illumina Nextseq500 using the Nextseq High Output 75 cycles kit using 2.6 pM of the pooled libraries. Around 20-30M single-end PF reads were generated per sample. Library preparation and sequencing were made at the IRIC Genomics Platform.

Bioinformatics

The RNA-seq data was run in a bioinformatics pipeline using the Compute Canada clusters (assigned to Dr. Gaël Moquin-Beaudry from Dr. Martine Tétreault's lab). The alignment was performed using HiSAT2 (382) against reference genome mm19. Read counts were obtained with htseq-count (383), and differential expression analysis was performed with the Bioconductor R package TCC v1.24.0 (384). Group data were analyzed pairwise and normalized using the DEGES/DESeq2 generalized linear model. Differentially expressed genes were identified with the TCC implementation of likelihood ratio test within DESeq2 with a p-value < 0.01 threshold. GO enrichment analysis on up-and down-regulated DEGs was performed using ShinyGO v0.61Ge SX, Jung D & Yao R, Bioinformatics 2020. For hierarchical clustering tree summarizing the correlation among significant pathways, the pathways with many shared genes are clustered together. Bigger dots indicate more significant p-values. The network plot also shows the relationship between enriched pathways. Two pathways (nodes) are connected if they share 20% or more genes. Darker nodes are more significantly enriched gene sets, bigger nodes represent larger gene sets, thicker edges represent more overlapped genes. Gene set analysis was accomplished on DEGs using GSEA 4.0.3 (385). Enriched gene sets were then curated from GSEA using the following keywords: lipids (lipid, fat, sterol), immunity (inflam-, cytokine, immun-,

neutro-, antigen, leukocyte, chemotaxis, lymphocyte, interleukin, myeloid, interferon, chemokine), and synapses (synap-, dendri-, neurotrans-, spine).

Golgi analysis

Golgi staining

To expose the hippocampus, mouse brains were cut into hemispheres and at 5mm from the olfactory bulb and 4 mm from the cerebellum with a mouse brain mold. For mice that received SCD1 inhibitor or vehicle, the contralateral hippocampus from osmotic pumps was used. Golgi staining was performed with the sliceGolgi kit (Bioenno Tech cat# 003760). Slices were post-fixed in fixative solution for 1 hour at 4°C and then in fresh fixative solution for an additional 48 hours at 4°C before slicing on a vibratome at 150um. Approximately 16 sections per animal were stained, between -1.06mm to -3.52mm from Bregma (n=4 animals per group). Briefly, sections were washed 3 times with 0.2M phosphate buffer pH 7.4. Free-floating sections were then stained in the impregnation solution for 24 hours and then in fresh impregnation solution for another 4 days at room temperature on a rocker. Then sections were washed once with phosphate buffer saline-Triton X (PBS-T) to remove impregnation solution. Subsequent washes were performed with PBS 1X pH 7.4. Golgi staining was performed by incubating sections in Solution C for 1 minute and then in post-staining solution D for 2 minutes. Sections were dehydrated in a series of ethanol baths (50%, 80%, 95%, and 100%) followed by two Xylene washes. Sections were then mounted onto slides with Permount (Thermofisher Scientific, cat# SP15-100) and allowed to dry overnight under a chemical hood before imaging.

Golgi quantification

Only neurons where the cell body and secondary and tertiary dendrites were completely visible were selected for quantification. The dorsal hippocampus sections were between -1.06mm to -2.70mm from Bregma while the ventral hippocampus sections were -2.70mm to -3.52mm from Bregma. Dendritic spine density was assessed by counting spines on a length of 19µm on secondary or tertiary dendrites of neurons in the dentate gyrus or the CA1 of the hippocampus on a light microscope at 100X (Olympus DP21). Quantification was performed on two neurons per region per section for a total of 24 neurons per group. For the analysis of dendritic arborization, Z stacks were taken at a step of 0.2µm at 40X on a light microscope (Olympus IX2-UCB). Stacks were analyzed with ImageJ v1.52p (386). Total dendritic length and Sholl analysis were assessed

with Fiji plug-in “Simple Neurite Tracer”. For Sholl analysis, the distance of concentric circles drawn from the middle of the cell body was set at 10µm.

Flow cytometry analysis

Brain single-cell dissociation

Single cells from the whole brain were achieved using the protocol established by Dr. Natalie Arbour’s and Dr. Catherine Larochelle’s labs. This was performed by Audrey Daigneault, Oumarou Ouédraogo and myself. Briefly, mice were perfused with 40ml of cold PBS 1X pH 7.4 (diluted from PBS 10X (Wisent, cat# 811-012-CL) with MilliQ water), brains were dissected and transferred to cold Hibernate media (Thermofisher Scientific, cat# A12475-01). To digest the tissue, brains were cut into small pieces with a scalpel, transferred to a tube containing 14µg/mL DNase I (Sigma Aldrich, cat# 10104159001) and 2mg/ml Collagenase D (Sigma Aldrich, cat# 11088866001) in Hank's balanced salt solution (HBSS) 1X (Wisent, cat# 311-512-CL), then placed in a 37ºC water bath with shaking for 15 minutes. Brain homogenates were then filtered through a 100µm cell strainer and centrifuged for 7minutres at 1400rpm at 4ºC. The supernatant was discarded, and the pellet was resuspended in Percoll 37% (Sigma Aldrich, cat# 17-0891-01) to remove myelin. Homogenates were centrifuged for 10 minutes at 1500 rpm without brakes to achieve phase separation, the pellet was washed with Hibernate media, and samples were centrifuged for 10 minutes at 1600 rpm at 4ºC. Finally, the supernatant was discarded, and the cell pellets were resuspended in Hibernate media with 1% fetal bovine serum (FBS) (Wisent, cat# 085150).

Flow cytometry staining

Cell staining was performed using the protocol established by members of Dr. Natalie Arbour’s and Dr. Catherine Larochelle’s labs, Audrey Daigneault and Oumarou Ouédraogo. Briefly, dissociated cells were counted with Trypan blue (Sigma Aldrich, cat# T8154) and 1 million cells were transferred into wells of a 96 wells plate with a V bottom. Cells were treated with 1 µg/ml Brefeldin A (Sigma Aldrich, cat# B7651) for 1 hour at 37ºC and washed with flow cytometry (FC) buffer (Hibernate (Thermofisher Scientific, cat# A12475-01) + 1% FBS (Wisent, cat# 085150)) by centrifuging samples at 1600rpm 7 minutes 4°C. The pellet was then resuspended with Aqua vivid (Thermofisher Scientific, cat# L34966), a live/dead stain for 30 minutes at 4°C, washed with FC buffer, and centrifuged at 1600rpm for 7 minutes at 4°C. Cells were then fixed and

permeabilized with FoxP3/Transcription Factor Fixation/Permeabilization solution (eBioscience, cat# 00-5521-00) for 30 minutes at 4°C, washed and again centrifuged at 1600rpm for 7 minutes at 4°C. Extracellular staining was achieved by incubating cells with the (Table 1) antibodies diluted in FC buffer for 20 minutes at 4°C, cells were then washed with FC buffer and centrifuged at 1600rpm for 7 minutes at 4°C. Next, cells were resuspended in 200µl FC buffer and analyzed on the BD LRS II (BD Biosciences) flow cytometer. For intracellular staining, cells were incubated with the (Table 1) antibodies diluted in FC buffer with Triton-X 0.1% (Fisher Scientific, cat# BP151100) overnight at 4°C, washed with FC buffer, and centrifuged at 1600rpm for 7 minutes 4°C. Cells were then resuspended in 200µl FC buffer and analyzed at the flow cytometer BD LRS II (BD Biosciences). Data was analyzed on FlowJo by Dr. Catherine Larochelle's and Dr. Natalie Arbour's labs. Flow cytometry analysis of microglia was performed by gating on CD45 intermediate- $CD11b^{+}$ cells.

Table 1. List of antibodies

Name	**Company (cat#)**	
Immunocytochemistry		**Dilution**
Alexa488 donkey anti-rabbit	Invitrogen (A21206)	1:1000
Alexa555 goat anti-mouse	Invitrogen (A21424)	1:1000
IBA1 (rabbit)	Wako (019-19741)	1:500
GFAP (mouse)	Chemicon (MAB360)	1:1000
Flow cytometry		**Volume per million cells**
Arg1 A700	eBioscience (56-3697-80)	2 µl
Aqua vivid	Thermofisher Scientific (L34966)	0.5 µl
CD11b BV605	BD Biosciences (563015)	0.5 µl
CD206 PE	Biolegend (141706)	2 µl
CD45 Cy7	Biolegend (103114)	2 µl
CD86 V450	BD Biosciences (560449)	2 µl
iNOS APC	eBioscience (17-5920-80)	2 µl
MHC II BV650	BD Biosciences (563415)	2 µl
TNFa PerCPCy5.5	BD Biosciences (560659)	1 µl

Primary microglia experiments

Primary microglia culture

This is a modified protocol from Dr. Thierry Alquier's lab. Primary microglia were obtained from whole brains of P1 WT or 3xTg pups. Following the dissection of the brain, meninges were carefully removed in an ice-cold solution of HBSS 1X without Ca^{2+} and Mg^{2+} (Wisent, cat# 311-512-CL). The brain was then cut into small pieced with a scalpel and filtered through 100µm cell strainer in Dulbecco's Modified Eagle Medium (DMEM), high glucose without sodium pyruvate (Invitrogen cat# 11965118) containing 10% heat deactivated FBS (Wisent cat# 085150) and 1% penicillin and streptomycin (P/S) (Wisent cat# 450-201-EL). Cells from 2 brains were resuspended in 12mL of media (DMEM+ %10 FBS+ 1%P/S) and cultured in a T-75 flask in a 37ºC incubator with 5% CO_2. After 3 days in culture, the media was changed to remove debris. The media was subsequently changed 2 times a week until microglia isolation. When astrocytes reached 95-100% confluence, between DIV 11-14, microglia were isolated by incubating flasks on a rocker for 1 hour at 37ºC with 5% CO_2. Microglia were then plated on a 48 well plate coated with poly-D-lysine (Sigma Aldrich, cat# P9404) at a cell density of 100 000 cells/well and incubated for 48 hours before the experiment to allow microglia to enter a "homeostatic/resting state".

Primary microglia treatments

Microglia were treated with 25ng/mL of LPS (Sigma Aldrich, cat# L4516) diluted in PBS 1X (Wisent, cat#311-010-CL), Aβ oligomers and monomers (see "*Preparation of Aβm and Aβo"*) or 5µM of SCD1 inhibitor (Abcam cat# ab142089) diluted in DMSO. Treatments had a duration of 6 hours in a 37ºC incubator with 5% CO_2 after which cells were lysed to isolate total RNA.

Immunocytochemistry

Immunocytochemistry was performed on chamber slides. Cells were plated in chamber slides coated with poly-D-lysine (Sigma Aldrich, cat# P9404) at a cell density of 50 000 cells/well. After 48 hours after plating cells, they were first washed with PBS 1X pH 7.4 (diluted from PBS 10X (Wisent, cat# 811-012-CL)) and fixed with 4% paraformaldehyde (PFA) for 15 minutes at room temperature. PFA was washed away with PBS 1X pH 7.4 and then cells were permeabilized with 0.2% PBS-T for 7.5 minutes. Cells were washed and non-specific antigens were blocked with a blocking solution of 0.5% bovine serum albumin (Bioshop, cat# ABL001) and 6% goat serum (Jackson Immuno Research, cat# 005-000-121) for 2 hours. The primary antibodies (Table 1) were

diluted in 1:1 blocking solution dilution and PBS 1X pH 7.4 and incubated overnight at 4°C in a humid box. Primary antibodies were washed away with PBS 1X pH 7.4 and the secondary antibodies (Table 1) were added. Cells were incubated with the secondary antibodies (Table 1) for 45 minutes and then with Hoechst 1:500 (Sigma Aldrich, cat# B2261). Slides were rinsed with 0.2M PB and mounted with Mowiol mounting medium (13% (w/v) polyvinyl alcohol and 2% (w/v) DABCO in 2:1 Tris-Base (pH 8.5): Glycerol).

Quantification of microglial purity

The purity of microglial cultures was evaluated by immunocytochemistry. We quantified the number of $IBA1^+$ microglia and $GFAP^+$ astrocytes in a field in each well of chamber slides (n=6) at 20X on a fluorescent microscope (Olympus IX2-UCB). Microglial purity was calculated by dividing the number of IBA^+ cells by the total number of cells counted.

Preparation of mAβ and AβO

To $A\beta_{1-42}$ peptides (BACHEM cat# 4014447) were dissolved in ice-cold HFIP (Sigma-Aldrich, cat# 105228) to a concentration of 1µM. HFIP was evaporated under a laminar flow hood at room temperature. The resulting Aβ films were stored at -80ºC for no longer than 6 months. Aβ monomers were prepared by dissolving peptides in DMSO (Sigma Aldrich, cat# D2650) and brought to a concentration of 500µM with sterile PBS 1X (Wisent, cat#311-010-CL). To obtain oligomers, Aβ monomers were incubated at 4ºC for 42 hours after which AβO was isolated by centrifugation at 12 000rpm for 15 minutes at 4ºC and recuperating the supernatant. Aβ products were confirmed by SDS-PAGE and Coomassie blue (*see below*).

Gel electrophoresis

Gel electrophoresis was performed with sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gels. The stacking gel contained 5% acrylamide, 0.5M Tris pH 6.8, 1% ammonium persulfate (APS), 1% SDS and 0.05% Tetramethylethylenediamine (TEMED) while the separating gel was composed of 15% acrylamide, 1.5M Tris pH 8.8, 1% APS, 1% SDS and 0.05% TEMED. 10µg of samples were diluted in 4X sample buffer (0.5M Tris-HCl, 10%v/v SDS, 40% v/v Glycerol, 5% v/v β-mercaptoethanol, and bromophenol blue) and completed to 40µL with MilliQ water. The samples were heated at 95 °C for 5 minutes. Samples were then transferred in 1.5mm-thick SDS-PAGE gels and were run at 80-120V.

Coomassie blue staining

SDS-PAGE gels were stained with a 0.04% Coomassie blue (Bio-Rad, cat# 161-0406) filtered solution diluted in 25% methanol and 10% glacial acetic acid for 1 hour at room temperature and a Destain I solution (40% ethanol and 10% glacial acetic acid for 30 minutes at room temperature. Gels were then left overnight with Destain II solution (20% ethanol and 7% glacial acetic acid) on a rocker at room temperature. For imaging, gels were transferred in MilliQ water and imaged with a ChemiDoc MP imaging system (Bio-Rad).

Real-time quantitative PCR analysis

RNA extraction and cDNA synthesis

Total RNA was extracted with TRIzol (Invitrogen cat# 15596018) and chloroform (Invitrogen cat# 15593031) according to the manufacturer's instructions. RNA was quantified using a NanoDrop™ 2000/2000c Spectrophotometer (Thermofisher Scientific). cDNA was prepared from 1µg of total RNA using a mix of 1X Reverse transcriptase buffer (from 10X stock: 1M Tris pH 8.3, 1M $MgCl_2$), 25 mM dNTP mix (Invitrogen cat# 10297-018), 0.5M Dithiothreitol (Invitrogen cat# R0861), 20U RNAse inhibitor (Invitrogen cat# N8080119) and 100U M-MLV Reverse Transcriptase (Invitrogen cat# 28025-013). Samples were incubated at 37ºC for 1 hour. cDNA was diluted to a final concentration of 4ng/µL in RNAse- DNase-free water (Wisent, cat# 809-115-CL).

Real-time quantitative PCR

Real-time quantitative PCR was performed using SensiFAST 2X SYBR Lo-ROX kit Maser mix (Bioline cat#BIO-94050) on the QuantStudio3 (Applied Biosystems). Primers (Table 2) were diluted in RNAse- DNase-free water (Wisent, cat# 809-115-CL) to 40µM. Gene expression of target mRNAs was calculated using the Comparative Ct method and was normalized to expression levels of the housekeeping gene *GAPDH*.

Table 2. Sequences of primers

IL1β Forward	GGTACATCAGCACCTCACAA
IL1β Reverse	TTAGAAACAGTCCAGCCCATAC
GAPDH Forward	AACAGCAACTCCCACTCTTC
GAPDH Reverse	CCTGTTGCTGTAGCCGTATT
SCD1 Forward	AGCGGTACTCACTGGCA
SCD1 Reverse	CCCTACGACAAGAACATTCAATC

TNFα Forward	CCTCTTCTCATTCCTGCTTGT
TNFα Reverse	TGGGAACTTCTCATCCCTTTG

Statistical analyses

Statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software). Two-tailed unpaired t-test paired Student's t-test and one-way ANOVA with Dunnett's multiple comparisons test was used as well as two-way ANOVA with Tukey's multiple comparisons test. Data are presented as means ± standard errors of the mean. Statistical tests performed are detailed in the legend for each figure. P-values less than 0.05 were considered statistically significant.

Results

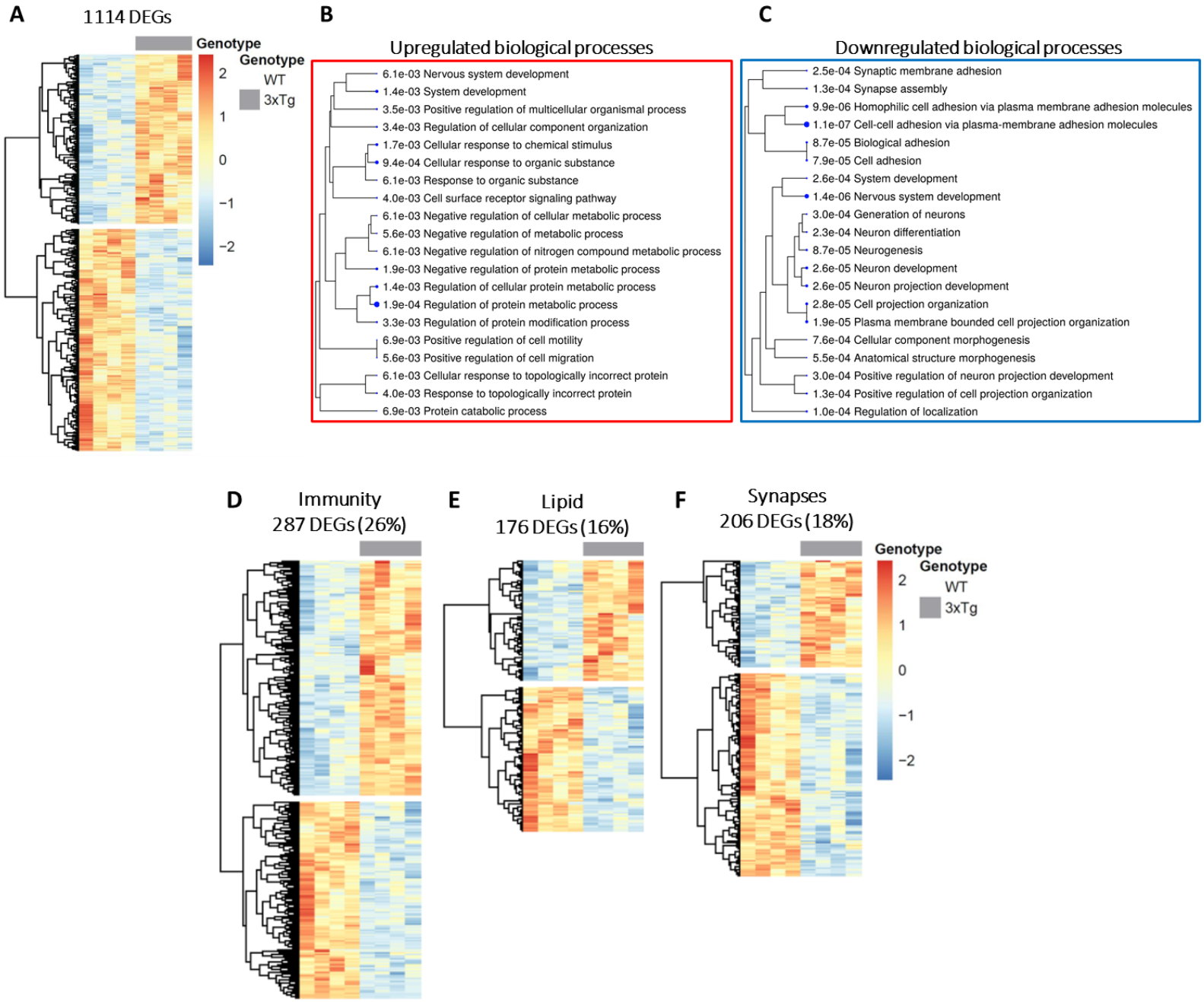


Figure 2. RNA-seq of WT and 3xTg hippocampus at 8 months of age. (A) Heatmap of z-score expression levels of genes found to be differentially expressed between WT and 3xTg hippocampus (n=4, p adj < 0.01). (B) GO enrichment analysis of biological processes that were found to be upregulated in the 3xTg hippocampus, n=4 (values correspond to enrichment false discovery rate (FDR)). (C) GO enrichment analysis of biological processes that were found to be

downregulated in the 3xTg hippocampus, n=4 (values correspond to enrichment FDR). (D) Heatmap of z-score expression levels of genes related to immunity found to be differentially expressed between the hippocampus of WT and 3xTg mice (n=4, p adj < 0.01). (E) Heatmap of z-score expression levels of genes related to lipids found to be differentially expressed between the hippocampus of WT and 3xTg mice (n=4, p adj < 0.01). (F) Heatmap of z-score expression levels of genes related to synapses found to be differentially expressed between the hippocampus of WT and 3xTg mice (n=4, p adj < 0.01).

Alterations in the transcriptome of the 3xTg hippocampus correspond to process altered in AD.

We investigated the disparities between the transcriptome of WT and 3xTg hippocampus at the early stages of AD when memory deficits are already present. To do this, we microdissected the whole hippocampus of middle-aged of 4 WT and 4 3xTg mice at 8-month of age at stage preceding plaque and tangle formation (379) and proceeded to perform RNA isolation with a TRIzol reagent. The concentration of RNA isolated was measured by UV spectrophotometry via a Nanodrop and a solution of 50µL of RNA at a concentration of 20ng/µL was sent to the IRIC genomic platform for sequencing with the Illumina Nextseq500 system. A bioinformatician Dr. Gaël Moquin-Beaudry from Dr. Martine Tetreault's lab assisted with the sequencing data.

We identified 1114 significantly differentially expressed genes (DEGs) between WT and 3xTg hippocampus with an adjusted p-value < 0.01 threshold (figure 2A). We performed gene ontology (GO) enrichment analysis of the 482 DEGs that were found to be upregulated and the 632 DEGs that were downregulated. This revealed that altered biological processes corresponded to process known to be altered in AD, including upregulation of cell metabolism and response to misfolded proteins (figure 2B) in addition to downregulation of synapses and neuronal differentiation (figure 2C). We were interested in assessing the proportions of DEGs that were associated with major processes that are modified in AD, such as immunity, lipids, and synapses. To do this, we closely studied the significant GO terms of biological processes provided by gene set enrichment analysis (GSEA) and searched for keywords associated with immunity, lipids, and synapses. We found that amongst the 1114 DEGs, 26% of DEGs were related to immunity (figure 2D), 16% related to lipid (figure 2E), and 18% related to synapses (figure 2F). Altogether, the transcriptome in the hippocampus of 3xTg mice displays changes in processes known to be altered in AD.

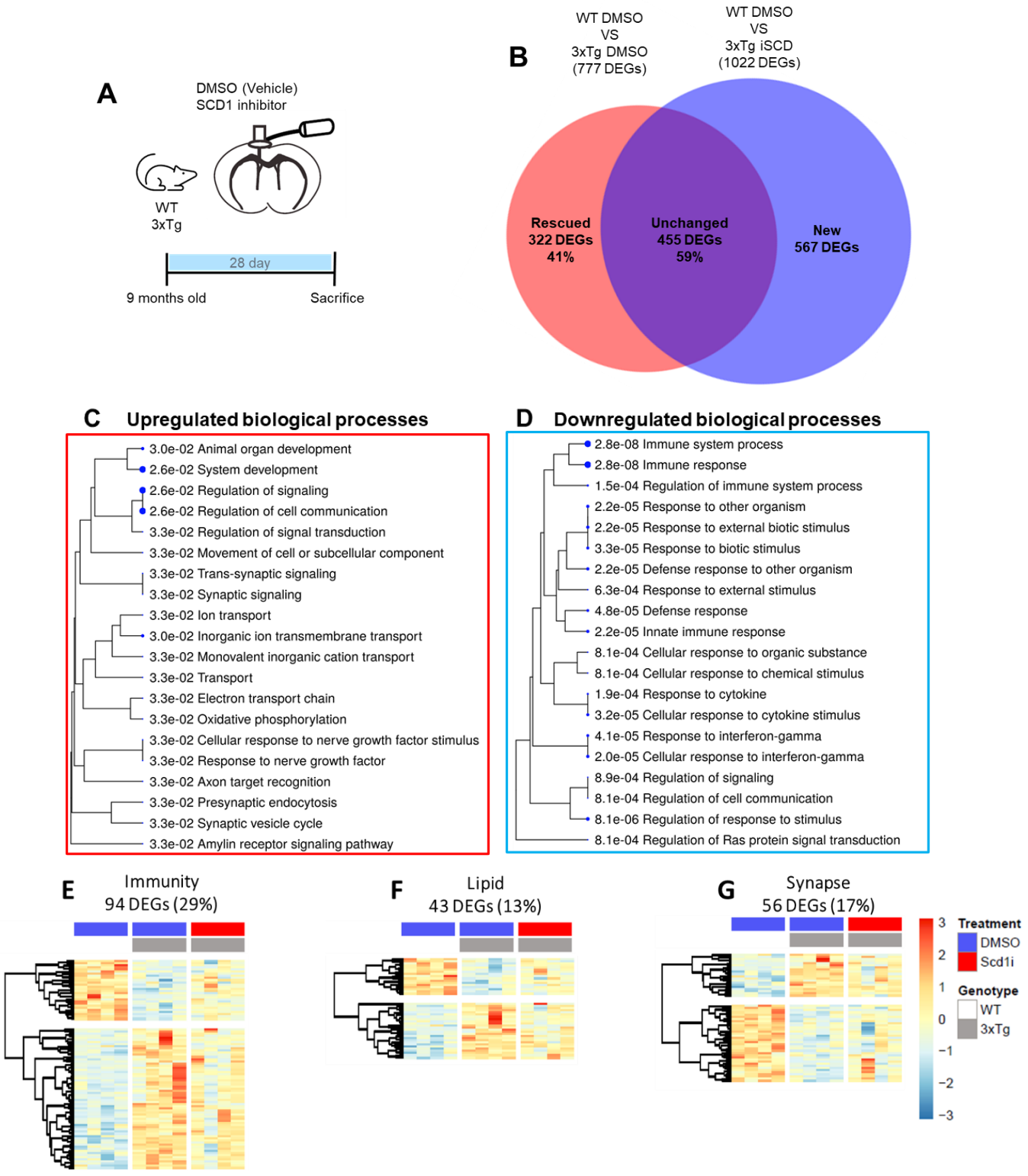


Figure 3. SCD1 inhibition rescued genes related to immunity and synapses in the 3xTg hippocampus. (A) Experimental design. (B) Venn diagram highlighting the effect of SCD1 inhibition on the transcriptome of the 3xTg hippocampus, n=4. (C) GO enrichment analysis of

rescued biological processes that were found to be upregulated in the 3xTg hippocampus, n=4 (values correspond to enrichment FDR). (D) GO enrichment analysis of rescued biological processes that were found to be downregulated in the 3xTg hippocampus, n=4 (values correspond to enrichment FDR). (E) Heatmap of z-score expression levels of genes related to immunity found to be rescued with SCD1 inhibition in the 3xTg hippocampus (n=4, p adj < 0.01). (F) Heatmap of z-score expression levels of genes related to lipids immunity found to be rescued with SCD1 inhibition in the 3xTg hippocampus (n=4, p adj < 0.01). (G) Heatmap of z-score expression levels of genes related to synapses found to be rescued with SCD1 inhibition in the 3xTg hippocampus (n=4, p adj < 0.01).

SCD1 inhibition rescued 41% of alterations in the 3xTg hippocampus.

To determine the effect of SCD1 inhibition on the transcriptome of the 3xTg hippocampus, we administered an SCD1 inhibitor or its vehicle, DMSO, in the brains of 9-month-old WT and 3xTg mice via intracerebroventricular infusion with a sample size of 4 mice per group (figure 3A). The administration of SCD1 inhibitor or DMSO through the ventricles allows for the distribution of the products throughout the brain by the CSF. After 28 days of treatment, we sacrificed mice and microdissected the whole hippocampus. We processed the hippocampus as previously mentioned.

We wanted to determine if treating 3xTg mice with SCD1 inhibitor normalizes alterations in their transcriptome to possess a similar gene expression to WT mice treated with the vehicle. We did a first comparison between WT and 3xTg mice treated with the vehicle. This led to the identification of 777 DEGs with an adjusted p-value < 0.01 cut-off (figure 3B). Then, we performed a second comparison between WT DMSO-treated mice and 3xTg mice treated with SCD1 inhibitor. This led to the identification of 1022 DEGs with a cut-off of p adj < 0.01 (figure 3B). We found that amongst the genes that were initially differently expressed between WT and 3xTg mice treated with DMSO, 322 DEGs were no longer significant between WT DMSO and 3xTg treated SCD1 inhibition (rescued) while 455 DEGs remained (unchanged) (figure 3B). This indicates that SCD1 inhibition normalized 41% of DEGs in the 3xTg hippocampus. SCD1 inhibitor also led to the appearance of 567 new DEGs (new) that were not initially found to be significant when comparing the hippocampal transcriptome of WT and 3xTg mice treated with DMSO (figure 3B). We further investigated the biological processes of genes that were rescued with SCD1 inhibition in the 3xTg hippocampus by GO enrichment analysis. Biological processes related to synapses were amongst

upregulated processes (figure 3C) while most downregulated processes were involved with immunity (figure 3D). Consistent with this, 29% of DEGs were associated with immunity (figure 3E) and 17% with synapses which together represent just under 50% of the rescued DEGs (figure 3G). A proportion of rescued genes were also related to lipids (figure 3F). These data suggest that SCD1 inhibition could be beneficial for rescuing AD changes related to immunity and synapses in the hippocampus.

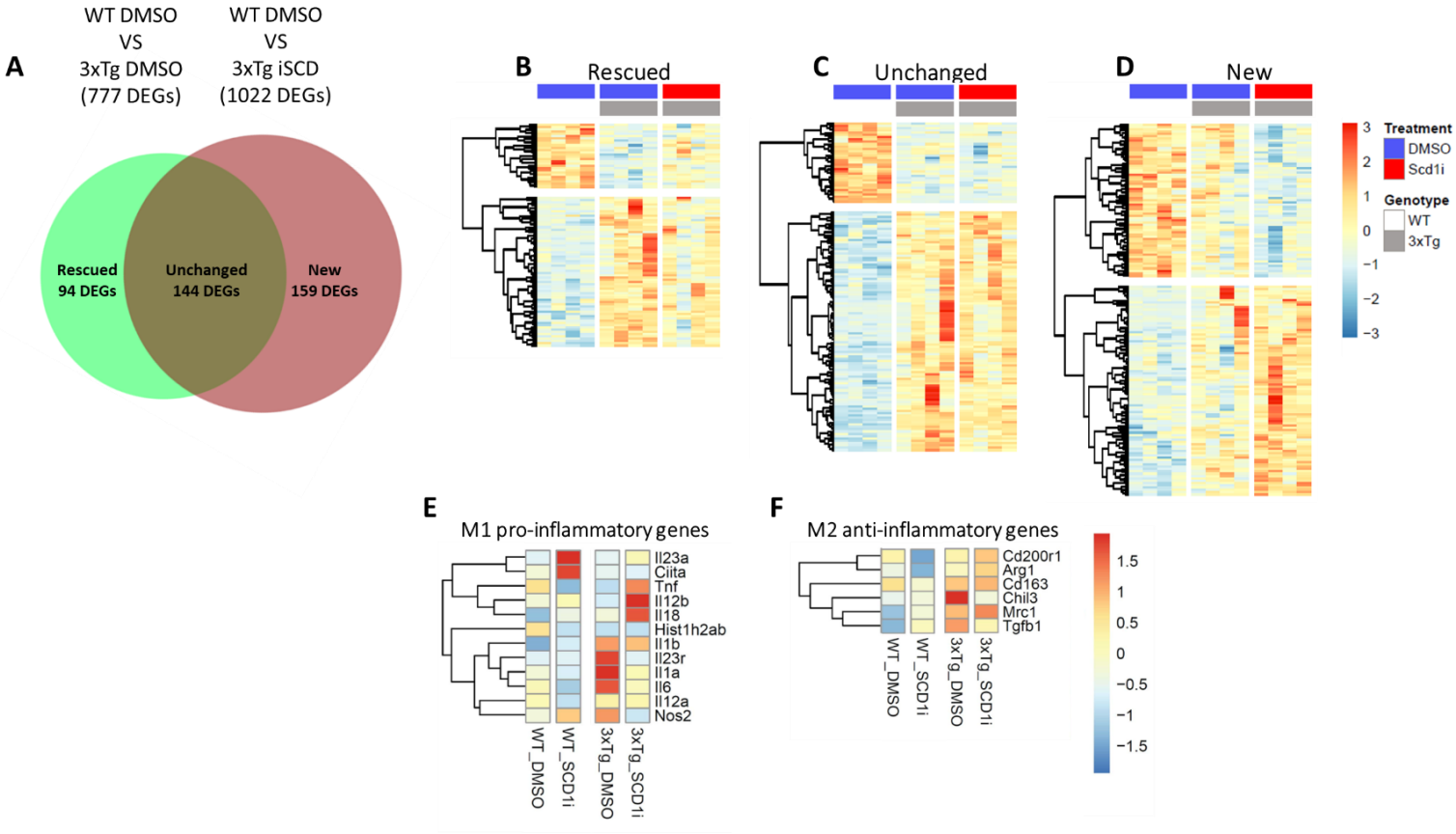


Figure 4. Effect of SCD1 inhibition on immune-related genes in the 3xTg hippocampus. (A) Venn diagram summarizing the effect of SCD1 inhibition on DEGs associated with immunity in the 3xTg hippocampus, n=4. (B) Heatmap of z-score expression levels of genes related to immunity found to be rescued with SCD1 inhibition in the 3xTg hippocampus (n=4, p adj < 0.01). (C) Heatmap of z-score expression levels of genes related to immunity found to be unchanged with SCD1 inhibition in the 3xTg hippocampus (n=4, p adj < 0.01). (D) Heatmap of z-score expression levels of genes related to immunity found to be new with SCD1 inhibition in the 3xTg hippocampus (n=4, p adj < 0.01). (E) Heatmap of z-score expression levels of genes associated

with M1 pro-inflammatory phenotype (n=4, p adj < 0.01). (F) Heatmap of z-score expression levels of genes associated with M2 anti-inflammatory phenotype (n=4, p adj < 0.01).

Effect of SCD1 inhibition on immune-related genes in the hippocampus.

We studied the effect of SCD1 inhibition on immune-related genes more closely and found that among the DEGs reported in the previous figure (figure 3B), 94 DEGs were rescued, 144 DEGs were unchanged and 159 new DEGs appeared with SCD1 inhibition (figure 4A-D). Pro-inflammatory markers have been reported to have increased levels in AD brains as anti-inflammatory markers were reduced (186, 387-389). Therefore, we also pulled out genes related to M1 and M2 phenotype to determine if SCD1 inhibition affects the polarization of immune cells. In general, in mice treated with DMSO, it seems that the 3xTg hippocampus upregulates both M1 pro-inflammatory and M2 anti-inflammatory genes in comparison to the WT hippocampus (figures 4E-F). SCD1 inhibition in the 3xTg hippocampus appears to alter gene expression of M1 genes by downregulating half of these genes (figure 4E). In contrast to M1 genes in the 3xTg hippocampus, SCD1 inhibition does not seem to affect M2 genes (figure 4F). Altogether, it seems that SCD1 inhibition affects the expression of M1 pro-inflammatory genes in the 3xTg hippocampus.

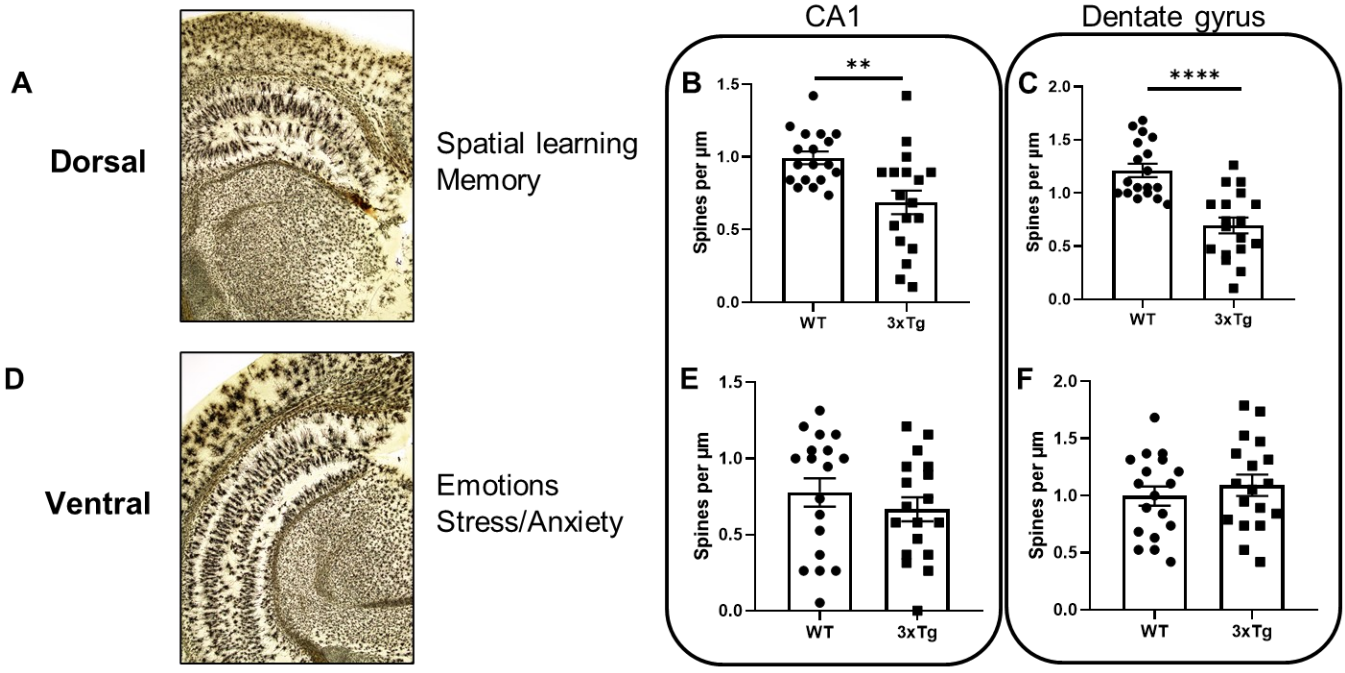


Figure 5. Dendritic spine loss occurs in the dorsal hippocampus of 3xTg mice at 8 months of age. (A) Representative 4X image of Golgi staining of the dorsal hippocampus. (B) Dendritic spine density in the CA1 of the dorsal hippocampus (n=18, t-test **p<0.01). (C) Dendritic spine density

in the dentate gyrus of the dorsal hippocampus (n=18, t-test **** p<0.0001). (D) Representative 4X image of Golgi staining of the ventral hippocampus. (E) Dendritic spine density in the CA1 of the ventral hippocampus (n=18, t-test). (F) Dendritic spine density in the dentate gyrus of the ventral hippocampus (n=18, t-test).

Dendritic spine loss occurs in the dorsal hippocampus of middle-aged 3xTg mice.

To assess, dendritic pathology in the 3xTg hippocampus, we performed Golgi staining. This method for staining neurons and their dendrites was established by Camillo Golgi which published his drawing in 1886 (390). Known for his drawings of neurons, Santiago Ramón y Cajal used the Golgi staining method to visualize isolated neurons and alongside Camillo Golgi, they won a Nobel prize in 1906 for their depictions of neuronal structures. We purchased a Golgi staining kit and modified its protocol to obtain quantifiable and isolated neurons. These parameters included cutting the brains in two to allow diffusion of the staining solutions and reducing the concentration of detergent for washes to allow uniformization of staining.

We evaluated dendritic spine density in hippocampal slices of pre-plaque middle-aged WT and 3xTg mice at 8 months of age. At this age, 3xTg mice display deficits in memory and increase anxiety (unpublished). We investigated the loss of dendritic spines of neurons in the cornu ammonis 1 (CA1) and the dentate gyrus of the hippocampus. The CA1 and the dentate gyrus are sub-regions of the hippocampus where atrophy was duly documented (391). We first measured in the dorsal hippocampus (figure 5A), a region associated with spatial learning and memory (392), and found significant spine loss on the dendrites of neurons in the CA1 (figure 5B) and dentate gyrus (figure 5C) of 3xTg mice when compared to WT mice. We were also interested in studying the ventral hippocampus (figure 5D) which is associated with emotions and anxiety (392). We did not measure a difference in the ventral hippocampus of 3xTg mice in the CA1 (figure 5E) nor in the dentate gyrus (figure 5F). These results show that the reduction of dendritic spine density occurs in the dorsal hippocampus in middle-aged 3xTg mice and not in the ventral hippocampus.

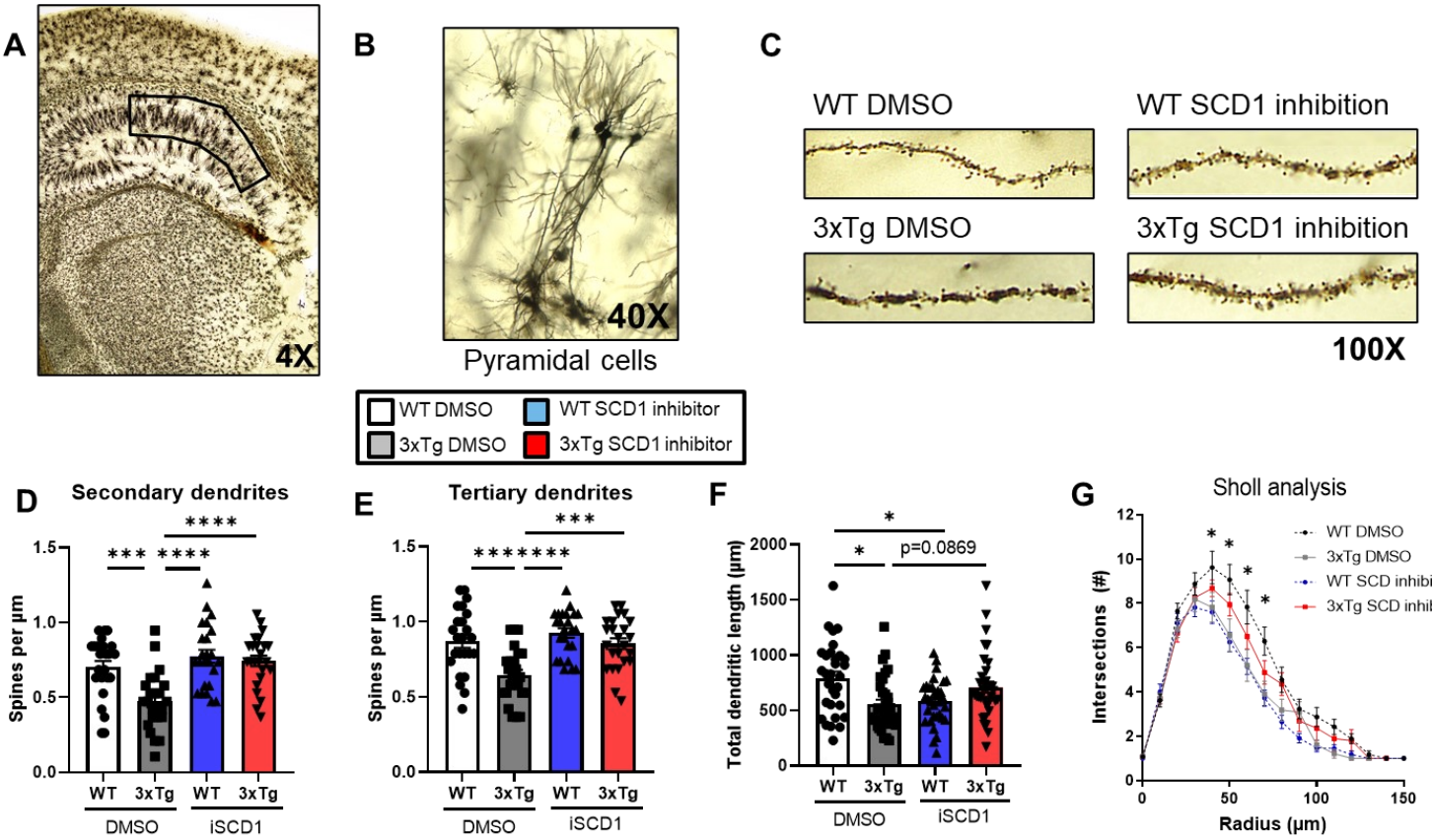


Figure 6. SCD1 inhibition rescued the loss of dendrites and spines in pyramidal cells of the CA1 in the 3xTg hippocampus (A) Representative 4X image of Golgi staining of the dorsal hippocampus with the black frame represents the CA1. (B) Representative 40X image of Golgi staining of pyramidal cells in the CA1. (C) Representative 100X image of Golgi staining of dendrites of pyramidal neurons. (D-E) Dendritic spine density of pyramidal cells in the CA1 of the dorsal hippocampus (n=24, two-way ANOVA ***p<0.001, ****p<0.0001). (F) Total length of dendrites of pyramidal neurons (n=35, two-way ANOVA *p<0.05). (G) Quantification of the number of dendritic intersections per circle set at 10µm by Sholl analysis (n=35, two-way ANOVA *p<0.05).

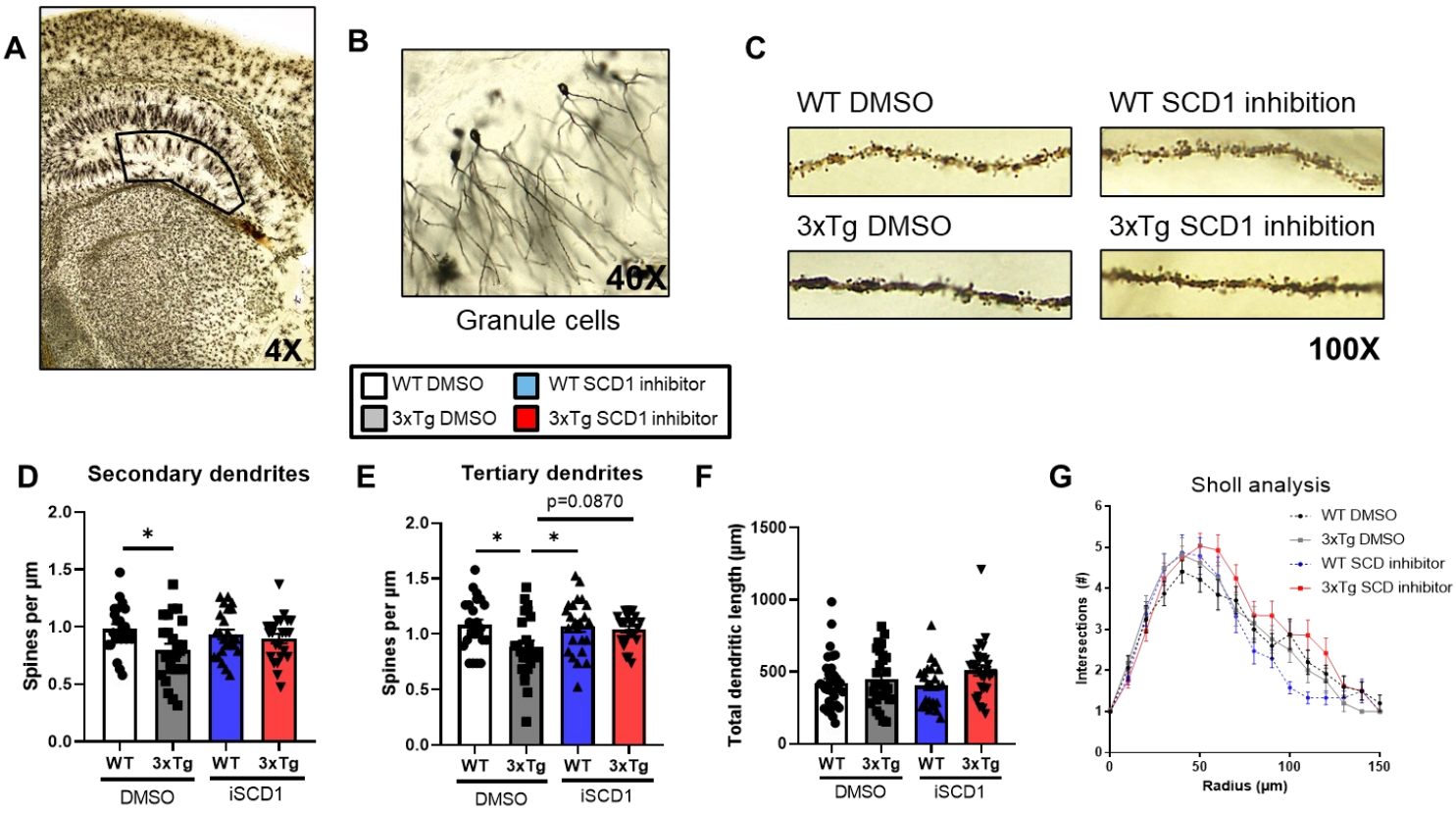


Figure 7. SCD1 inhibition did not rescue the loss of spines in granule cells of the dentate gyrus in the 3xTg hippocampus (A) Representative 4X image of Golgi staining of the dorsal hippocampus with the black frame represents the dentate gyrus. (B) Representative 40X image of Golgi staining of granule cells in the dentate gyrus. (C) Representative 100X image of Golgi staining of dendrites of granule cells. (D-E) Dendritic spine density of granule cells in the dentate gyrus of the dorsal hippocampus (n=24, two-way ANOVA *p<0.05). (F) Total length of dendrites of granule neurons (n=35, two-way ANOVA). (G) Quantification of the number of dendritic intersections per circle set at 10µm by Sholl analysis (n=35, two-way ANOVA).

SCD1 inhibition rescued the loss of dendrites and spines in the 3xTg hippocampus.

Since SCD1 inhibition rescued synapse-related genes (figure 3C and 3G) as well as memory in 3xTg mice (unpublished), we investigated its effect on dendritic arborization and spine density in the dorsal hippocampus of 3xTg mice. We hypothesized that SCD1 inhibition would ameliorate neurite pathology in the hippocampus of 3xTg. To verify this hypothesis, we performed Golgi staining on slices of the hippocampus of 9-month-old WT and 3xTg mice whose brain was infused with the SCD1 inhibitor or its vehicle, DMSO, for 28 days (figure 3A). We measured total

dendrites length and quantified dendritic spine density of neurons in the CA1 region (figure 6A) and the dentate gyrus (figure 7A). We also performed Sholl analysis on the dendritic tree to assess dendritic complexity. This consists of quantifying the number of intersections between dendrites and concentric circles drawn from the middle of the cell body and distanced at 10µm from each circle.

In the CA1 region, we studied apical dendrites of pyramidal neurons (figure 6B) and their dendritic spines (figure 6C). When comparing vehicle-treated mice, dendritic spine density on the secondary (figure 6D) and tertiary dendrites (figure 6E) of 3xTg mice was significantly reduced. The inhibition of SCD1 rescued spine loss in 3xTg mice (figure 6D, 6E). We measured dendritic projections and examined the dendritic complexity of neurons in the CA1. We found that dendrites were shortened in 3xTg mice treated with DMSO (figure 6F) which also displayed reduced complexity of dendrite branching compared to WT DMSO mice (figure 6G). A recovery of dendritic pathology was observed following SCD1 inhibition in 3xTg mice (figure 6F, 6G). Interestingly, SCD1 inhibition resulted in a reduction of dendritic projection length (figure 6F) and dendritic complexity (figure 6G) in WT mice.

We performed similar analyses on the granule cells of the dentate gyrus (figure 7B). We assessed dendritic spine density (figure 7C) and found that this was also significantly reduced in the dentate gyrus of 3xTg mice compared to WT mice (figure 7D, 7E). Spine loss on secondary dendrites was not recovered with SCD1 inhibition 3xTg mice although there was so significant difference between WT mice treated with DMSO and 3xTg mice treated with SCD1 inhibitor (figure 7D). On tertiary dendrites, though it did not result in a significant effect, SCD1 inhibition seems to slightly stimulate dendritic spine growth in 3xTg mice (figure 7E). These data suggest that SCD1 inhibition promotes spine growth to a lesser extent to the CA1. As for dendritic arborization of neurons within the dentate gyrus, we did not observe a significant difference in dendritic projection length (figure 7F) or dendritic complexity (figure 7G) between WT and 3xTg mice treated with DMSO and nor did SCD1 inhibition in both strains affect the dendritic tree in the dentate gyrus (figure 7F-G). However, although insignificant, SCD1 inhibition did seem to have a positive effect on dendritic projection length (figure 7F) or dendritic complexity (figure 7G).

Together, these results indicate that aberrant lipid metabolism contributes to dendrite and spine loss in 3xTg mice and that SCD1 inhibition allows for recovery of loss of synapses and some possible benefits on the dendritic tree in the CA1 and the dentate gyrus of these mice.

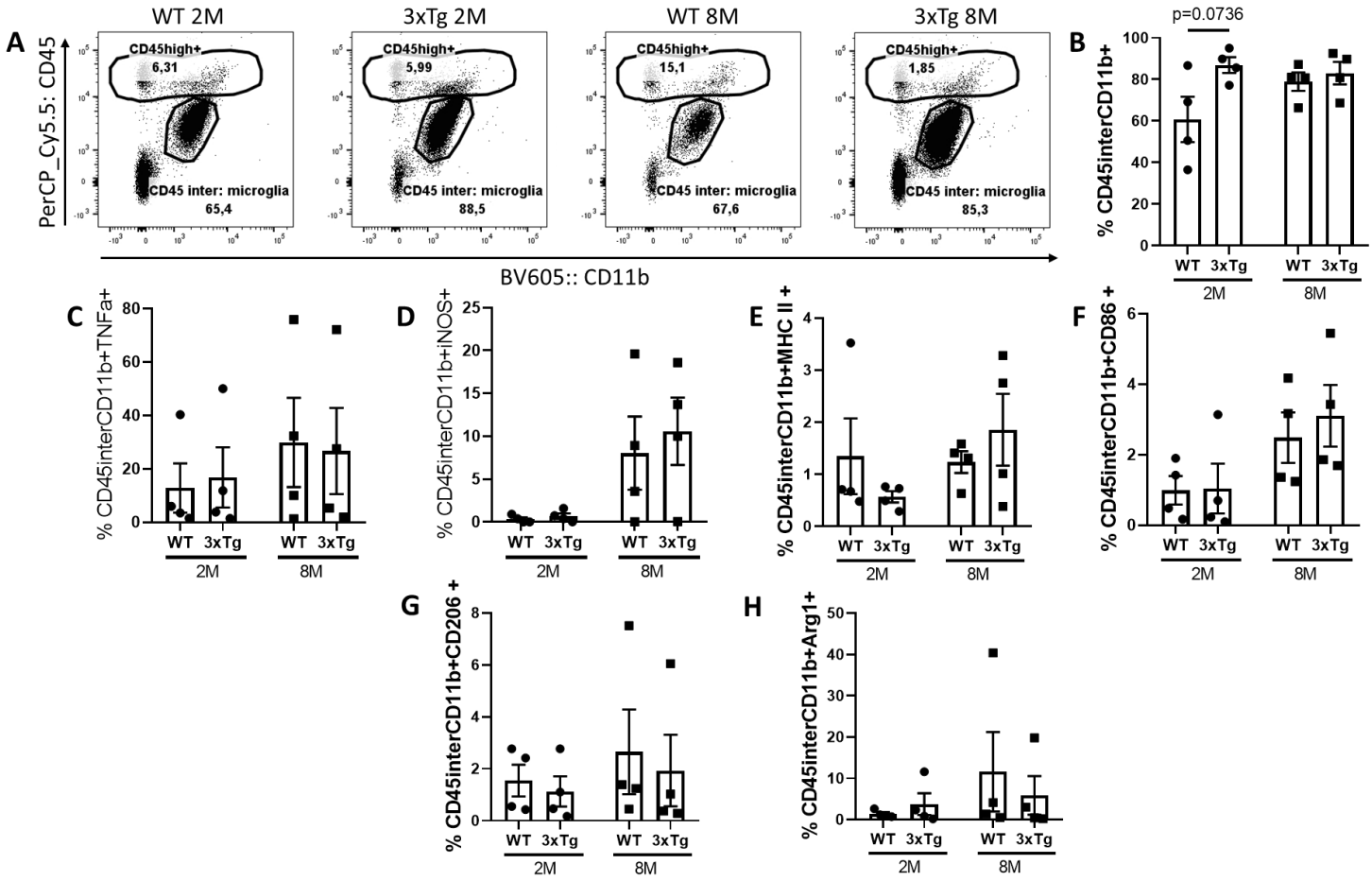
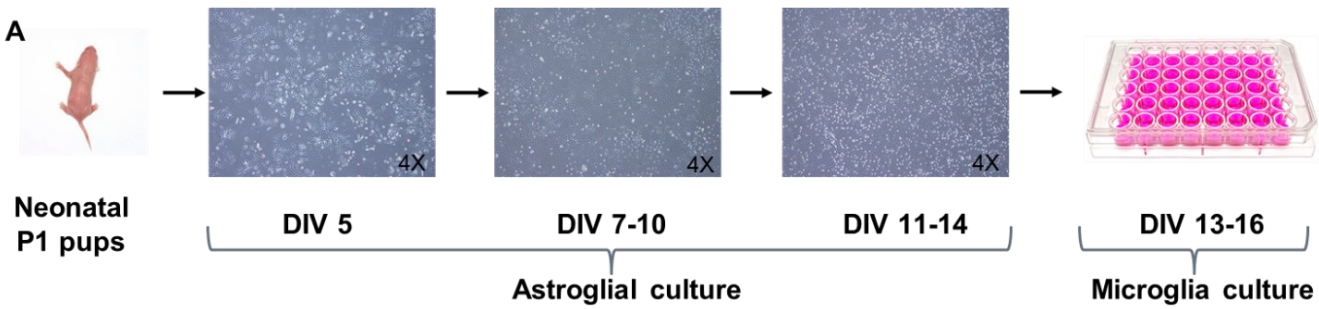


Figure 8. Characterization of microglial polarization in the brain of asymptomatic and symptomatic mice. (A) Gating strategy for microglia. (B) Proportion of microglia amongst immune cells in the brain (n=4, two-way ANOVA). (C-F) Proportion of microglia in the brain that expresses pro-inflammatory markers TNFα, iNOS, MHC II, and CD86, respectively (n=4, two-way ANOVA). (G-H) Proportion of microglia in the brain that expresses anti-inflammatory markers CD206 and Arg1, respectively (n=4, two-way ANOVA).

<u>3xTg brains seem to display high proportions of microglia as early as 2 months of age.</u>

In AD, microglia are known to adopt a pro-inflammatory phenotype which often is accompanied by deleterious effects on the brain. To better understand microglial behavior at the early stages of AD, we compared the proportion of M1 pro-inflammatory and M2 anti-inflammatory microglia in

WT and 3xTg brains at a young/asymptomatic age and early stages in AD. To do this, we performed flow cytometry on the whole brains of 2- and 8-month-old WT and 3xTg mice in collaboration with the labs of Dre Catherine Larochelle and Dre Natalie Arbour. Flow cytometry analysis of microglia was performed by gating on CD45 intermediate/medium-CD11b^{+} cells while CD45 high cells represent macrophages (figure 8A). We studied the proportion of microglia in the brains of mice and characterized microglial phenotype with markers associated with M1 pro-inflammatory microglia (TNFα, inducible NO synthase (iNOS), MHC II and CD86) or M2 anti-inflammatory microglia (CD206 and Arg1). In this pilot study, we observed a significant amount of variability, which may be technical or due to inter-animal variability. Nevertheless, certain observations can be made. We did not observe a significant difference between WT and 3xTg microglia at both ages. However, at 2 months of age, 3xTg brains seem to possess a higher proportion of microglia than WT mice and this effect was absent in 8-month-old mice (figure 8B). Additionally, there appears to be a trend following age in the proportion of WT and 3xTg microglia expressing pro-inflammatory markers (figure 8C-F). Aging is associated with increased inflammation in a phenomenon known as "inflammaging" (23, 272). Interestingly, the proportion of 3xTg microglia expressing anti-inflammatory cytokines (figure 8G-H) seems to be reduced compared to WT microglia at 8 months of age. This would coincide with findings that in AD, microglia downregulated genes associated with homeostatic and anti-inflammatory microglia AD (8, 9, 30). However, further work needs to be done to confirm these observations and to duly characterize microglial polarization in 3xTg throughout AD pathology.



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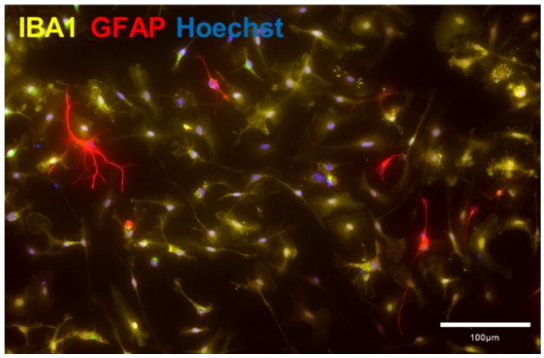

C

Chamber	#IBA⁺ cells	#GFAP⁺ cells	Purity (%)	
1	407	3	98.57	98.3%
2	251	4	98.43	
3	159	1	99.38	
4	143	3	97.95	
5	151	2	98.69	
6	208	7	98.31	

Figure 9. Establishment of a protocol for primary microglia culture. (A) Protocol to culture microglia from an astroglial culture from the brains of neonatal pups. (B) Immunocytochemistry of cells found in the resulting primary microglia culture (yellow: IBA1 microglia, red: GFAP astrocyte, blue: Hoechst stain; scale bar 100µm). (C) Quantification of microglia IBA1^{+} cells and astrocyte GFAP^{+} cells to assess the purity of microglia culture.

Establishment of a protocol for primary microglia culture.

Microglia culture is a useful tool to study microglia-specific inflammatory responses. In general, primary microglia cultures from rodents come from neonatal animals as adult microglia are difficult to sustain in culture (393). We initially obtained the protocol from Dr. Thierry Alquier's lab and we modified it to match the requirements of our experiments. The optimized protocol starts with the whole brains of P1 neonatal WT and 3xTg pups (figure 9A). Our protocol consists of isolating the brains of P1 pups and culturing a mixed culture of dissociated single cells. We use media with high glucose and supplemented it with FBS to promote astrocyte and microglia proliferation. In vitro astrocytes are crucial for microglial survival and proliferation as they provide microglia with factors such as colony-stimulating factor, cholesterol, and TGFβ (251). After 5 days in vitro (DIV), we perform the first media change to remove debris and maintain the culture until astrocytes reach confluence around 7 to 10 days in culture. When astrocytes reach confluence, there is an increasing number of round bright cells, microglia, which appear around DIV11-14 and

this is when microglia are ready to be harvested. We harvest microglia by placing the flask on a shaker in an incubator causing microglia to detach and to be found in suspension while astrocytes stay attached. Microglia are then plated in a 48 well plate at a density of 100 000 cells per well. We assessed the purity of our primary microglia culture by plating microglia on chamber slides and performing immunocytochemistry for microglia ionized calcium-binding adaptor molecule 1 (IBA1) $^{+}$ cells and astrocytes glial fibrillary acidic protein (GFAP) $^{+}$ cells (figure 9B). We counted the microglia IBA1^{+} cells and astrocytes GFAP^{+} cell in subfields on each chamber resulting in an average purity of 98.6%.

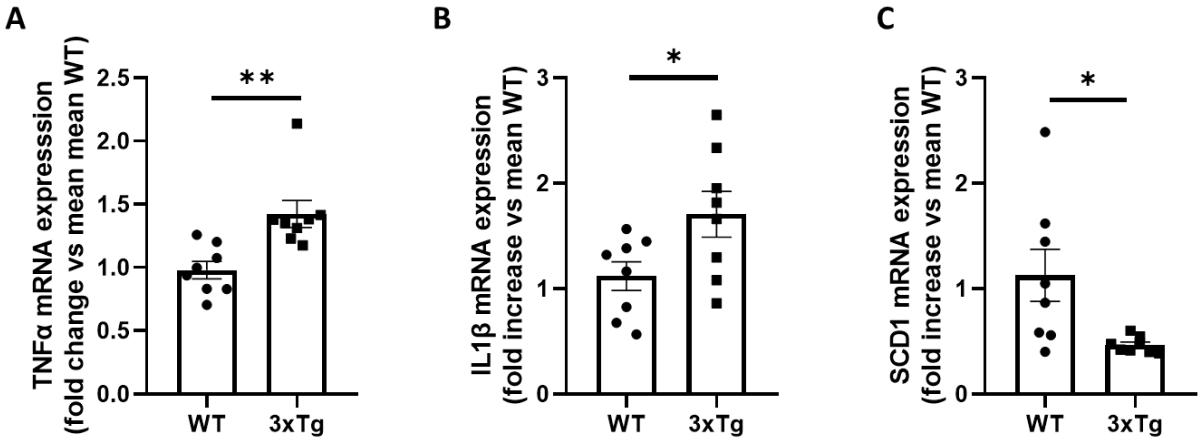

Figure 10. 3xTg microglia have higher mRNA levels of pro-inflammatory cytokines at baseline. (A-B) mRNA expression of pro-inflammatory cytokines TNFα and IL1β, respectively (n=8, t-test, ** p<0.01). (C) mRNA expression of SCD1 (n=8, t-test, **** p<0.0001). The housekeeping gene used is GAPDH.

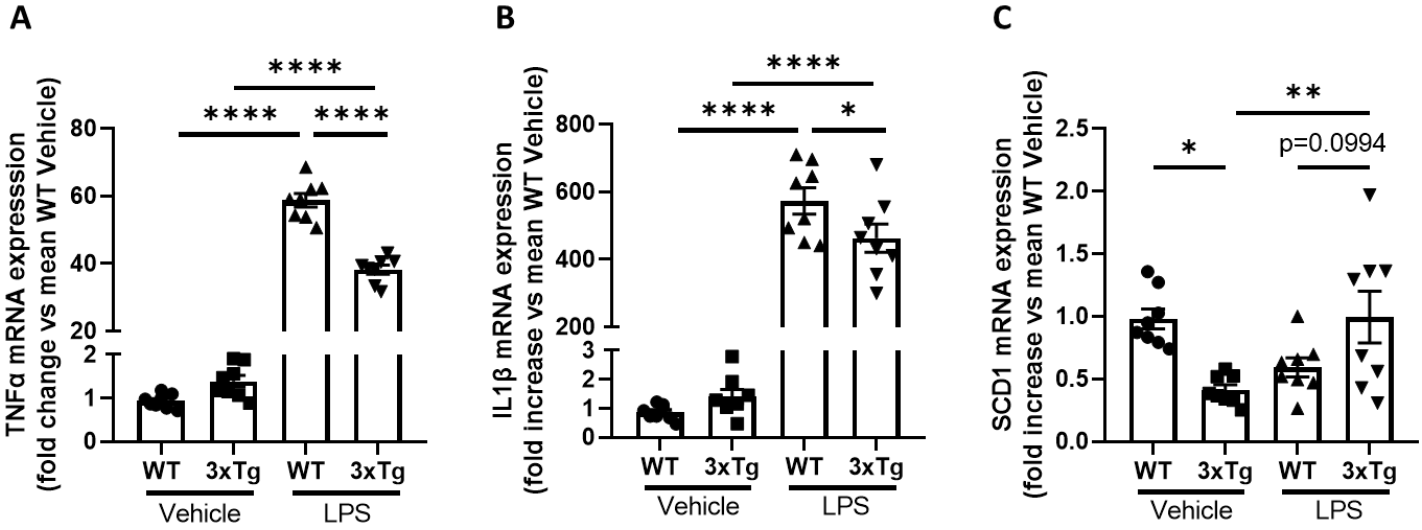

Figure 11. 3xTg microglia display a dampened response to LPS. (A-B) mRNA expression of pro-inflammatory cytokines TNFα and IL1β, respectively, in response to exposure to LPS (n=8, t-test, * $p<0.05$, **$p<0.01$). (C) mRNA expression of SCD1 in response to exposure to LPS (n=8, t-test, * $p<0.05$). The housekeeping gene used is GAPDH.

3xTg microglia express increased mRNA levels of pro-inflammatory cytokines but display a dampened response when challenged.

We compared the expression of pro-inflammatory cytokines, TNFα and IL1β, in WT and 3xTg microglia at baseline and found that 3xTg microglia express higher mRNA levels of TNFα (figure 10A) and IL1β (figure 10B) than WT microglia. We also observed that 3xTg microglia had a decreased mRNA expression of SCD1 compared to WT microglia (figure 10C). When challenged with a strong inducer of inflammation, acute 6-hour treatment of LPS at a concentration of 25ng/mL, 3xTg microglia exhibit a blunted response displayed by a reduced expression of TNFα (figure 11A) and IL1β (figure 11B) as opposed to WT microglia. It is important to note that when treated with the vehicle (PBS 1X), the significant increased expression of pro-inflammatory were no longer observed although it seems to be slightly elevated (figure 11A-B). Interestingly, LPS-induced inflammatory response resulted in a normalization of SCD1 expression in 3xTg microglia to WT microglia levels. These results imply that 3xTg microglia in culture exhibit a similar behavior than what is observed in AD where microglia upregulate pro-inflammatory cytokines and possess a reduced ability to respond and to engage an immune response. Additionally, pro-inflammatory LPS increased the expression of SCD1 within 3xTg microglia to resemble WT microglia levels. This suggests that microglia-mediated inflammation might be responsible for the rise in SCD1 in AD brains.

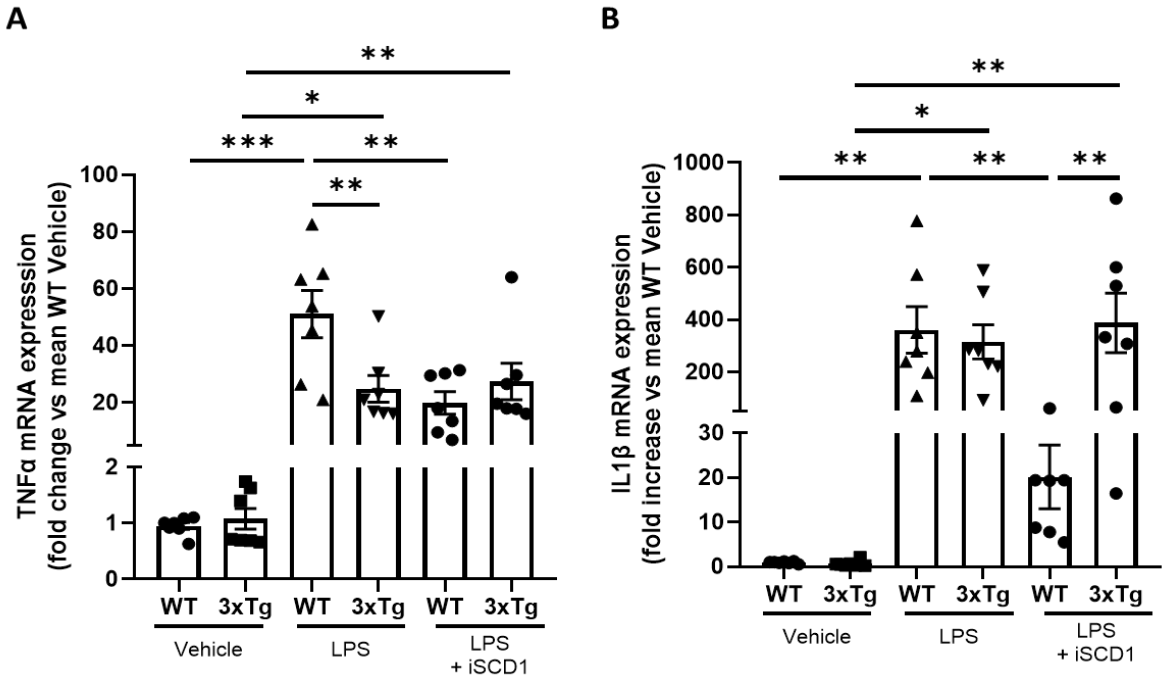

Figure 12. SCD1 inhibition does not reduce mRNA levels of pro-inflammatory cytokines in 3xTg microglia. (A-B) mRNA expression of pro-inflammatory cytokines TNFα and IL1β, respectively, in response to exposure to LPS and SCD1 inhibitor (n=7, two-way ANOVA, * $p<0.05$, ** $p<0.01$). The housekeeping gene used is GAPDH.

SCD1 inhibition does not reduce the mRNA expression of pro-inflammatory cytokines in 3xTg microglia.

As our RNA-seq data show that inflammation is downregulated with SCD1 inhibitor (figure 3D-E), we investigated the effect of SCD1 inhibition on microglia-mediated inflammation. We treated WT and 3xTg microglia with LPS and an SCD1 inhibitor for 6 hours and observed that SCD1 inhibitor has a significant effect on inflammation in WT microglia but not on 3xTg microglia which is partially consistent with results observed with RNA-seq of their hippocampus where SCD1 inhibition increase TNFα gene expression while IL1β expression seemed unchanged (figure 4E). SCD1 inhibitor partially blunted the expression of TNFα (figure 12A) and IL1β (figure 12B) in WT treated with LPS. However, it did not affect the expression of pro-inflammatory cytokines by 3xTg microglia (figure 12A-B). Of note, similar to the previous figure, no significant difference was observed between WT and 3xTg microglia treated vehicle, PBS 1X+ DMSO (figure 12A-B). These results suggest that SCD1 inhibition does not mediate inflammation by acting through pro-inflammatory cytokines in 3xTg microglia.

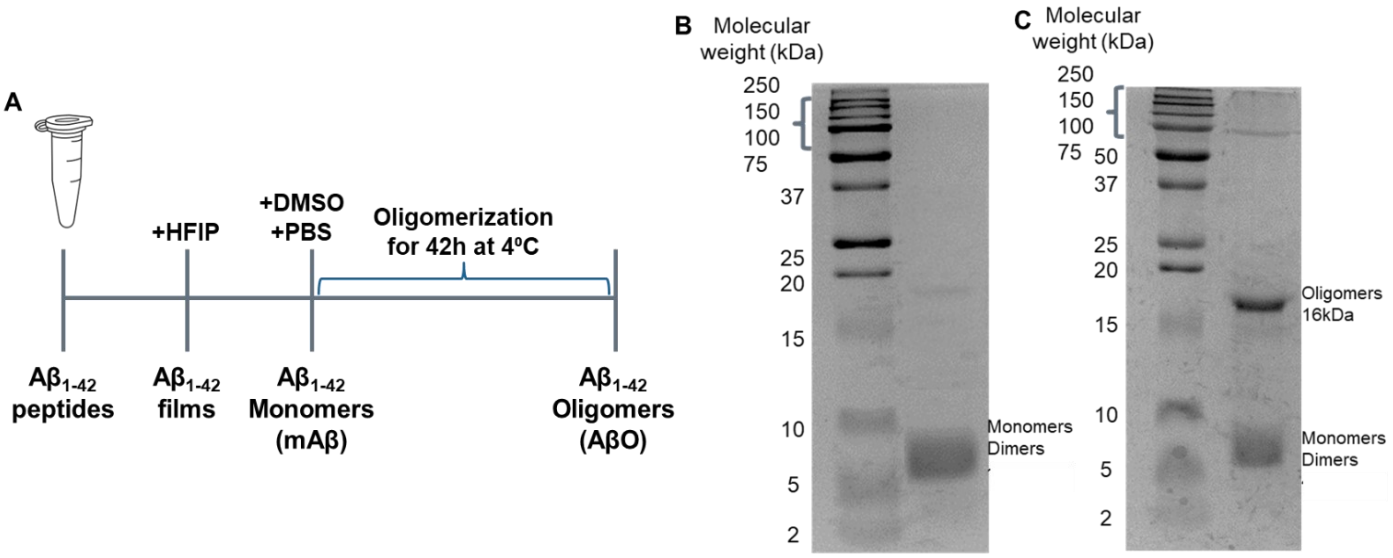

Figure 13. Preparation of $A\beta_{1-42}$ oligomers. (A) Protocol for $A\beta_{1-42}$ oligomerization. (B) Coomassie blue staining of an SDS-PAGE gel of monomeric Aβ preparation. (C) Coomassie blue staining of an SDS-PAGE gel of oligomeric Aβ preparation.

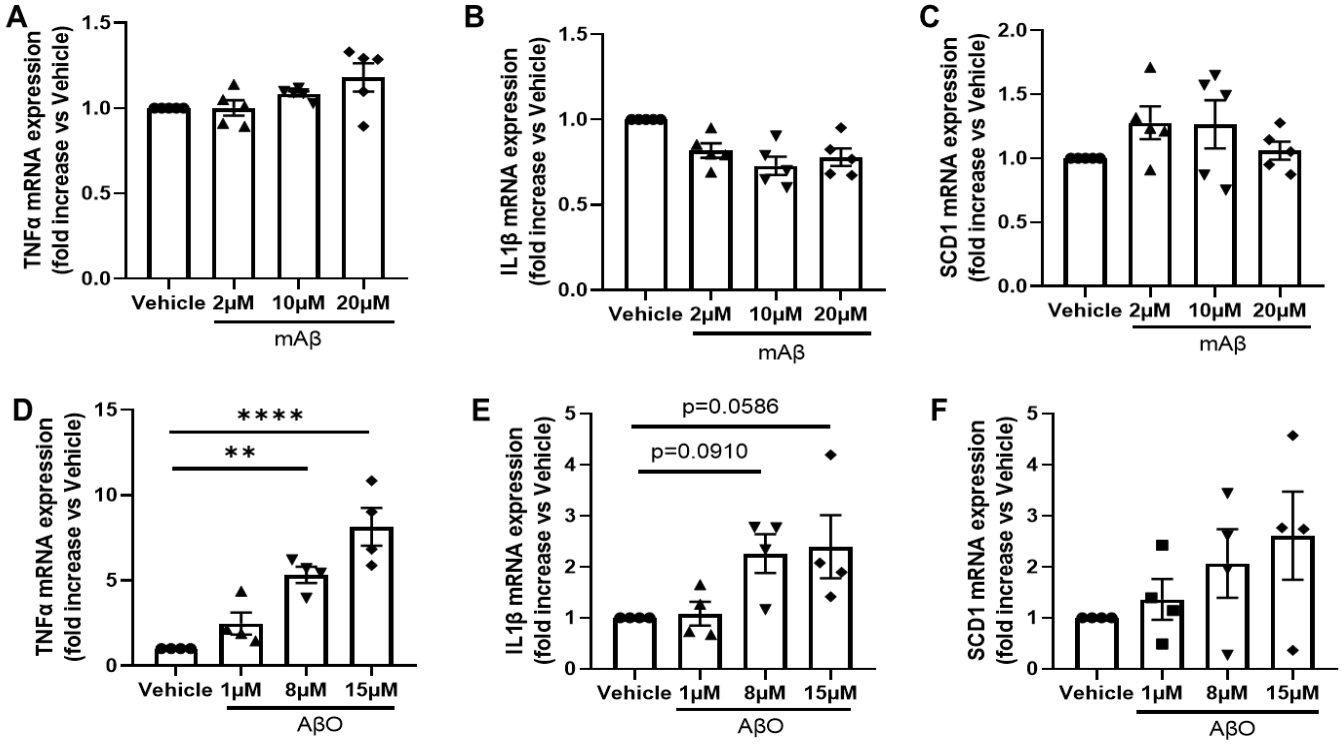

Figure 14. AβO-induced inflammation increases SCD1 mRNA expression in WT microglia. (A-B) mRNA expression of pro-inflammatory cytokines TNFα and IL1β, respectively, in response to exposure to mAβ (n=5, one-way ANOVA). (C) mRNA expression of SCD1 in response to

exposure to mAβ (n=5, one-way ANOVA). (D-E) mRNA expression of pro-inflammatory cytokines TNFα and IL1β, respectively, in response to exposure to AβO (n=4, one-way ANOVA, **p<0.01, **** p<0.0001). (F) mRNA expression of SCD1 in response to exposure to AβO (n=4, one-way ANOVA). The housekeeping gene used is GAPDH.

AβO-induced inflammation might be responsible for the upregulation of SCD1 in microglia.

The increase in SCD1 expression with LPS treatment suggests that inflammation is related to lipid metabolism (figure 11C). We investigated an inflammatory molecule relevant to AD, Aβ. AβO is the most neurotoxic (69) and potent inflammatory species of Aβ (69, 73, 74) of Aβ. Therefore, we treated WT microglia with Aβ monomers (mAβ) and AβO to determine the effect of Aβ on inflammation and SCD1 expression in "healthy" microglia to determine if we can replicate AβO-induced inflammation. Also, as a study reported that SCD1 was upregulated in macrophages (370), we wanted to study if this occurs in microglia as well. We establish a protocol to produce AβO (figure 13A). Monomeric and oligomeric Aβ are prepared from Aβ1-42 peptides which were solubilized with hexafluoroisopropanol (HFIP) and aliquoted to obtain Aβ films (figure 13A). Monomers were obtained from dissolving Aβ films with DMSO and diluted with PBS. As for oligomers, they were produced by incubating monomers at 4ºC for 42 hours to allow oligomerization. We confirmed the form of Aβ produce by migrating samples on an SDS-PAGE gel and staining the gel with Coomassie blue. mAβ possesses a molecular weight is around 4.5kDa (figure 13B) while AβO weighs over 16kDa (figure 13C).

We acutely treated WT microglia with increasing concentrations of mAβ and AβO for 6 hours. In microglia treated with mAβ, we did not observe an inflammatory response (figure 14A-B), nor did it lead to changes in the level of SCD1 mRNA expression (figure 14C). In microglia treated with AβO, levels of TNFα mRNA expression and concentrations of AβO displayed a proportional relation (figure 14D). As for IL1β and SCD1 expression, there seems to be a trend towards increase expression with increasing concentrations of AβO although it did not attain significance (figure 14E-F). This experiment needs to be replicated. However, this suggests that inflammatory Aβ species, AβO, might be responsible for SCD1 upregulation in AD.

Discussion

There is currently no cure for AD as the mechanism underlying the development and progression of this disease remains unclear. Consequently, clinical trials are struggling to find a cure for AD

as they test multiple hypotheses (15, 16). Studies into the genetics of AD have identified alterations in pathways implicated in immune cell response, lipid metabolism as well as amyloid and tau proteins influencing APP processing and Aβ production in both SAD-LOAD and FAD-EOAD (1-4). As studies have greatly focused on amyloid and tau pathologies along with inflammation, lipid metabolism has been omitted. Previous work in our lab has shown lipid metabolism abnormalities in AD human and mouse brains and we have observed that SCD1 inhibition had benefits in early loss of neurogenesis (104) and cognitive deficits (unpublished).

The purpose of my project was to investigate SCD1 inhibition as a therapeutic approach for intervention in AD. We hypothesized that Aβ causes alterations in lipid metabolism at the early stages of AD which is responsible for changes in microglia immune response and other downstream effects in AD and that SCD inhibition can intervene in these processes (figure 15). In this memoir, we employed an SCD1 inhibitor to dissect the impact of abnormal lipid metabolism on AD pathology. Our results suggest that it modulates processes involved with neuronal projections and immunity. Altogether, these findings emphasize the important role of altered lipid metabolism in AD.

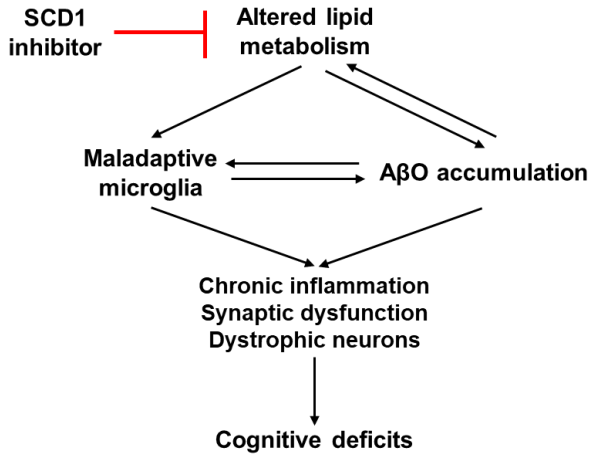

Figure 15. A hypothetical sequence of the pathogenetic steps of AD.

3xTg mouse model of Alzheimer's disease

We have to chosen to use 3xTg mice to conduct our experiments as it is the only mouse model that recapitulates the components of AD physiopathology. Nevertheless, it does have its constraints. The majority of our studies were performed on female 3xTg mice. Although 2-in-3 people with AD are women (10), this does not reflect the reality of AD as both male and female are affected

by this neurodegenerative disease. However, the absence of AD pathology in 3xTg male limits their practicality. Moreover, we did not consider the oestrus cycle of mice when performing our experiments. The oestrus cycle was reported to influence behavior (394, 395), synaptic plasticity (396, 397) and neuroinflammation (398, 399). Also, as hormonal fluctuations are a risk factor for AD, the stage of oestrus cycle may affect observed results. Additionally, the predominant form of AD is SAD-LOAD while 3xTg mice as well as other models of AD correspond to FAD-EAOD. A mouse model of SAD-LOAD is yet to be developed. Indeed, the incorporation of AD risk genes, *APOE ε4* allele or TREM2, in mice enhance the risk of SAD-LOAD but developing this form of AD also relies on the interplay of environmental and lifestyle risk factors in addition to a genetic component which is challenging to recreate.

The general consensus is that Aβ is responsible for initiating AD pathology. Our hypothesis is that Aβ pathology increases the activity of SCD leading to abnormal lipid metabolism and downstream effects in AD. If this hypothesis is true, we expect to obtain similar results in other mouse models of AD that exhibit Aβ pathology. As Fanning et al report that SCD inhibition inhibited α-synuclein aggregates and neuronal degeneration in a model of Parkinson's disease, the link between abnormal protein aggregation in neurodegenerative diseases and aberrant lipid metabolism warrants further investigation.

SCD1 inhibition rescued genes associated with immunity and synapses in the 3xTg hippocampus.

Our RNA-seq data of the hippocampus revealed changes in lipid metabolism, immunity, and synapses in 3xTg mice. These findings are similar to observations made by GWAS studies (ref). SCD1 inhibition rescued 41% of DEGs in which close to half of the normalized genes were associated with immunity and synapses. We also found that SCD1 could be modulating pro-inflammatory signals. The administration of the SCD1 inhibitor or its vehicle was done by installing an osmotic pump with a tube piercing the brain to be released in the lateral ventricle of mice. This procedure creates acute inflammation in the brain. However, with a treatment period of 28 days, this inflammation would have subsided at the time of sacrifice. Nonetheless, this is an important factor to consider as we are interested in inflammation in the brain. Moreover, the data we present in my memoir is quite superficial and mainly focused on rescued processes in the 3xTg hippocampus. We are currently investigating the processes that remained unchanged as well as the

new processes with SCD1 inhibition. Also, we have yet to explore the individual genes that were found to be differentially expressed. In particular, we must closely look into lipid-related genes to understand the beneficial effects of SCD1 inhibition. Furthermore, SCD1 inhibition could be altering the transcriptome in other brain regions. For instance, the cortex, another key region affected in AD, and the hypothalamus, the regulator of the endocrine system, would be potential prospects.

Our studies on gene expression were performed by bulk RNA-seq on the hippocampus. However, this technique possesses limitations. Bulk RNA-seq allows identifying gene expression patterns and global processes that are modified in pathologies. The caveat of this type of sequencing is that it relies on the average gene expression across a large number of cells that have been mashed up altogether. In a homogeneous tissue, the average gene expression is more likely to be represented as opposed to a highly heterogeneous tissue, such as the brain. Hence, microglia, which represent up to 12% of the brain cell population, will be outweighed by major cell populations, such as neurons and astrocytes. To circumvent this, we are currently working on single-cell RNA-seq (scRNA-seq) data from the brains of mice that received an SCD1 inhibitor. scRNA-seq will allow us to discern the cell-specific effect of SCD1 inhibition on the brain of 3xTg mice. Moreover, though we are mainly interested in microglia, SCD1 inhibition could be affecting other cells that regulate lipids in the brain, such as astrocytes and oligodendrocytes. However, studying gene expression through mRNA is not directly equivalent to protein levels. Proteomics can be performed to provide additional information on the levels and the modifications of proteins in pathological conditions.

SCD1 inhibition promotes regeneration of dendrites and dendritic spines in the 3xTg hippocampus.

We observed that middle-aged 3xTg mice displayed a reduction of dendritic spine density in the dorsal hippocampus but not in the ventral hippocampus. As the dorsal hippocampus is responsible for memory, this coincides with the appearance of cognitive symptoms of AD where memory deficits are the earliest symptom of AD. Alternatively, larger sample size may be required to observe a significant loss of dendritic spines in the ventral hippocampus of 3xTg mice. Our data also show that dendritic shortening and spine loss occur in their dorsal hippocampus in middle-aged 3xTg mice. SCD1 inhibition rescued changes in gene expression related to synapses as well

as dendrite and spine loss in the CA1 of the hippocampus of 3xTg mice. Although SCD1 inhibition did not have a significant effect on the dendrites of the dentate gyrus, it seemed to normalized spine loss as observed by the loss of significance between the spine density between WT mice treated with the vehicle and 3xTg mice treated with the SCD1 inhibitor. Additional work to increase the sample size might overcome these results. On the other hand, we did not see a difference in dendritic length between our experimental groups hinting that dendritic shortening occurs in the CA1 of the hippocampus before the dentate gyrus in middle-aged mice. Work done in our lab observed that SCD1 inhibition ameliorated memory and learning in 3xTg mice (unpublished). These findings imply that abnormal lipid metabolism plays a role in the loss of synapses and that SCD1 inhibition rescued cognitive deficits in 3xTg mice by promoting dendrite and spine regeneration although how SCD1 inhibition rescued dendrite and spine regeneration in the 3xTg hippocampus remains unknown. Additional work studying the functionality of synapses with electrophysiological approaches following SCD1 inhibition should be explored. This can be done by measuring long-term potentiation and -depression in the hippocampus of mice that received SCD1 inhibitor or the vehicle via an osmotic pump for 28 days or, on hippocampal slices.

Golgi staining is a commonly used technique to visualize dendrites and their spines. However, the disadvantage of this method is that it only stains a subset of neurons in a random manner by which the mechanism of selection is not yet understood. Also, the protocol of Golgi staining was rather arduous to optimize. We purchased a kit for Golgi staining, but we had to change parameters to achieve uniform staining without overstaining sections to obtain isolated neurons. This silver staining method allows studying spine morphology. Long and thin spines are associated with immature spines while mushroom-shaped spines are known as mature spines (400). This would have been interesting to study in our experiments. However, due to limited resolution on the microscope, we were not able to distinguish these categories of spines.

Microglial polarization in the 3xTg brain.

Flow cytometry is a helpful tool to characterize a population of cells in heterogeneous samples and to study the expression of molecules in these cells. In our experiment, we studied microglia in the brains of WT and 3xTg mice at two stages of AD, asymptomatic (2 months old) and symptomatic (8 months old). From the data we obtained from our pilot study with a sample size of 4 mice per

group, we were not able to conclude due to the high variability within experimental groups. This experiment needs to be further replicated to obtain a sufficient sample size to correct for variability.

However, this flow cytometry does have its caveats. As we wish to study microglial activation, it is important to note that the protocol for cell isolation from whole brains leads to the stimulation of microglia as they are highly sensitive to changes in their environment. An alternative to studying microglial activation in the brain is through immunohistochemistry. This would allow a spatial evaluation of microglia polarization in different brain regions. However, due to limitations in the number of allocated fluorescent channels, only a few proteins of interest can be studied at once. The advantage of flow cytometry in the presence of multiple laser channels allowing for up to 18 colors depending on cytometers. Thus, a larger panel of immune markers can be analyzed at once.

Establishment of a model of microglia to study cell-specific inflammatory responses.

Our experiments on primary microglia cultures demonstrate that 3xTg microglia express higher mRNA levels of pro-inflammatory cytokines but are unable to mount a proper immune response when challenged. Although, microglia treated with the vehicle no longer displayed significant differences between WT and 3xTg, the trend seemed to be conserved. Microglia are highly sensitive to the slightest changes in their environment. The evaluation of gene expression of microglia at baseline was performed without the addition of additives to the media while the vehicle contained PBS 1X and DMSO. This important factor should be considered. We observed that the acute activation of a pro-inflammatory response by LPS had a normalizing effect on SCD1 mRNA levels in 3xTg microglia. Additional studies looking into the effect of chronic inflammation on SCD1 expression should be investigated. As for AβO, we would need to increase our sample size, but our preliminary data suggest that inflammatory AβO was able to increase SCD1 expression in WT microglia. Although we have yet to treat 3xTg microglia with AβO, this suggests that AβO-induced activation of microglia is implicated in altered lipid metabolism in microglia. Hence, we expect 3xTg microglia to react similarly to WT microglia but with a higher expression on SCD1. It would also be interesting to treat microglia with Aβ fibrils to study the effect on SCD1 expression. As Aβ fibrils are less immunogenic as AβO, we anticipate an increase in SCD1 but at a lesser extent compared to AβO. In our in vitro studies, we have used a higher concentration of AβO than the ranges of concentration quantified in the brains of AD patients,

between picomolar to nanomolar concentrations, as a higher concentration of synthetic Aβ is required to obtain a similar effect (401-403).

Since our data on microglia culture rely on the expression of mRNA levels, we should verify the capacity of microglia to produce cytokines by Western blot and the secretory ability of microglia by measuring cytokines by enzyme-linked immunosorbent assay in the media of treated microglia. To test whether AβO is responsible for altering lipid metabolism in microglia, future approaches should by measuring glycolysis and OXPHOS by performing real-time metabolic analysis with the Agilent Seahorse machine which measures mitochondrial respiration. Furthermore, we still are studying the impact of SCD1 knockout on microglia immune response. This will provide additional insights into the role of SCD1 in inflammation.

Although additional work is necessary to support our hypothesis, microglia culture is a useful tool to study microglia-specific inflammatory responses in 3xTg mice, but it does have its caveats. Our protocol utilizes neonatal microglia which is widely accepted in the field. However, they lack the cellular interactions with other cells in the brain, such as astrocytes, neurons, and oligodendrocytes, and the exposure to changes occurring in the brain with aging and with the AD pathology. These components cause alterations in the epigenetics and the transcriptome of microglia which shape their functions and their behavior in pathologies. Efforts towards establishing a protocol to obtain microglia from adult brains could relieve this problem. Nonetheless, our data on primary microglia culture provide essential information regarding the link between microglia-mediated inflammation and lipid metabolism in AD.

Important questions left to answer.

Altogether, these findings show that targeting metabolic dysregulation through SCD1 inhibition could be a potential target for treating AD. However, as lipids are vital for brain function and SCD1 plays a central role in lipogenesis, determining alterations in the compositions of lipids in the brain with SCD1 inhibition is essential to understanding how this is beneficial for AD. As AD brains display alterations in energy consumption (404), we are also looking into the effect of SCD1 inhibition on the uptake of glucose and fatty acids in the different brain regions of WT and 3xTg with radioactive tracers, [18]- Fluorodeoxyglucose and 14(R, S)-[18F] Fluoro-6-thia-heptadecanoic acid, respectively. Be that as it may, our understanding of the mechanisms causing

AD-induced perturbations in lipid metabolism is shallow although it seems to play a central role in AD development and progression.

Conclusion

Disturbances in cell metabolism, lipid metabolism, in particular, is emerging as a central player in neurodegenerative diseases. In addition to work done in our lab, SCD1 has been gaining interest as a novel drug target for synucleinopathies, such as Parkinson's disease and dementia with Lewy bodies (368) and multiple sclerosis (369). Also, identifying the cause of neurodegenerative disease-induced perturbations in lipid metabolism is crucial to develop targeted therapeutical strategies. Future work focusing on altered lipid metabolism in the brain can provide leads to identifying new therapeutical strategies to alleviate cognitive deficits and improve metabolic deficits during AD.

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